BCL-XL directly modulates RAS signalling to favour cancer cell stemness

Citation
Carné Trécesson, S. d., F. Souazé, A. Basseville, A. Bernard, J. Pécot, J. Lopez, M. Bessou, et al. 2017. “BCL-XL directly modulates RAS signalling to favour cancer cell stemness.” Nature Communications 8 (1): 1123. doi:10.1038/s41467-017-01079-1. http://dx.doi.org/10.1038/s41467-017-01079-1.

Published Version
doi:10.1038/s41467-017-01079-1

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:34492434

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
BCL-X_L directly modulates RAS signalling to favour cancer cell stemness

Sophie de Carné Trécesson1,8, Frédérique Souazé1, Agnès Basseville1, Anne-Charlotte Bernard1, Jessie Pécot1, Jonathan Lopez2, Margaux Bessou2, Kristopher A. Sarosiek3, Anthony Letai3, Sophie Barillé-Nion1, Isabelle Valo4,5, Olivier Coqueret5,6, Catherine Guette5,6, Mario Campone7, Fabien Gautier1,7 & Philippe Paul Juin1,7

In tumours, accumulation of chemoresistant cells that express high levels of anti-apoptotic proteins such as BCL-X_L is thought to result from the counter selection of sensitive, low expresser clones during progression and/or initial treatment. We herein show that BCL-X_L expression is selectively advantageous to cancer cell populations even in the absence of pro-apoptotic pressure. In transformed human mammary epithelial cells BCL-X_L favours full activation of signalling downstream of constitutively active RAS with which it interacts in a BH4-dependent manner. Comparative proteomic analysis and functional assays indicate that this is critical for RAS-induced expression of stemness regulators and maintenance of a cancer initiating cell (CIC) phenotype. Resistant cancer cells thus arise from a positive selection driven by BCL-X_L modulation of RAS-induced self-renewal, and during which apoptotic resistance is not necessarily the directly selected trait.
Anti-apoptotic proteins of the BCL-2 family (BCL-2, BCL-X<sub>L</sub>, or MCL-1) are frequently up-regulated in cancers as a result of genetic, epigenetic or signalling pathway changes. BCL-2 homologues negatively regulate mitochondrial outer membrane permeabilisation (MOMP) and promote cell survival by counteracting death signals that result from direct activation of their pro-apoptotic multi-domain counterparts (BAX/BAK) by «activator» BH3-only proteins (BIM, BID or PUMA). They do so by sequestering the BH3 domains of pro-(BAX/BAK) by «activator» BH3-only proteins (BIM, BID or PUMA). Mechanisms that drive the outgrowth of high BCL-X<sub>L</sub> expressing cells are not fully characterized. The current consensus is that BCL-X<sub>L</sub> provides a survival advantage to cancer cells under apoptotic pressures induced discontinuously by therapy or continuously by oncogenic alterations. MOMP is indeed the primary way by which cancer cells die in response to radiotherapy, chemotherapy and to diverse stress stimuli cancer cells encounter as tumours progress. MOMP is also part of an intrinsic tumour suppressor mechanism induced by oncogenic alterations that lead to aberrant expression of C-MYC or loss of the pRB tumour suppressor<sup>3</sup>. These types of aberrations impose a sustained cell-autonomous pressure that should select cancer cells with higher levels of BCL-X<sub>L</sub>. However, not all oncogenic signals increase the apoptotic load of cancer cells and in some cases oncogene activity alleviates it instead. RAS activity for instance inhibits apoptosis<sup>4</sup>.

RAS pathway activation frequently occurs in solid tumours as a result of direct RAS mutations or of other less direct causes e.g. downstream of EGFR stimulation/activation. Importantly, RAS activity features have been described in the absence of RAS mutations in triple negative breast cancers. Activation of RAS and its downstream pathways MAPK/ERK and PI3K/AKT have well documented anti-apoptotic consequences due to the induction of anti-apoptotic proteins expression and the down-regulation or inactivation of pro-apoptotic effectors. The latter effect should decrease the pressure to select for cancer cells with enhanced expression of BCL-2 homologs. This raises the question of what, if any, selective advantage BCL-X<sub>L</sub> overexpression brings to cancer cells in RAS-activated tumours, and more generally in tumours that are not in receipt of an apoptotic pressure.

In addition to survival maintenance, other biological effects have been reported for BCL-X<sub>L</sub> and ascribed to its ability to interact with proteins beyond the BCL-2 family. BCL-X<sub>L</sub> may thus positively regulate biological functions contributing to tumour growth and dissemination by modulating the activity of some components of its vast interactome. How critical and advantageous such regulations would be to RAS-driven cancer cells and the binding partners involved in this context remain largely unknown. Dysregulated RAS activation induces a plethora of signalling pathways that favour cell proliferation, motility and invasion. In mammary epithelial cells, it promotes an epithelial to mesenchymal transition (EMT) and the emergence of cancer initiating cells (CICs) endowed with self-renewal capacities. CICs regenerate new tumours after an initial regression and play a critical role in tumour progression, in particular after treatment, to which they resist better than non-CICs. The influence of RAS activity on phenotypic plasticity and on the dynamic equilibrium between non-CICs and CICs therefore plays a key role in the expansion of epithelial tumour cell populations, initially or after relapse. We show that BCL-X<sub>L</sub> contributes to this process by interacting directly with RAS and fine-tuning its downstream activity.

**Results**

BCL-X<sub>L</sub> is required for RAS-induced CIC phenotype. To explore the biological functions of BCL-X<sub>L</sub> in transformed epithelial cells we used mammary epithelial MCF10A cells stably transduced with KRAS<sup>V12</sup> cDNA retroviral vectors. These cells are endowed with enhanced phenotypic plasticity and CIC properties. Indeed, MCF10A KRAS<sup>V12</sup> cells express mesenchymal markers (Supplementary Fig. 1a, e) and a subset of these cells form mammospheres and express enhanced levels of the typical CD44 marker (Supplementary Fig. 1b, c. Please also see Supplementary Methods and Supplementary Note 1 for more details). MCF10A KRAS<sup>V12</sup> cells exhibit a decrease in BCL2L11 mRNA (BIM) expression and enhanced BCL2L11 mRNA expression compared to controls (Fig. 1a). BCR3 (PUMA), PMAIP1 (NOXA), BAX or BAK mRNAs were in contrast expressed to similar levels in our matched pair of cell lines and BCL2, BCL2L2 and BCL2A1 expressions were barely detectable. BCL2L11 and BCL2L11 expressions in MCF10A KRAS<sup>V12</sup> cells are regulated by ongoing RAS activity as judged by the effects of RAF inhibition on their expression (Fig. 1b). Western blot analysis showed no detectable change in MCL-1 proteins levels, perhaps owing to the innate lability of this protein. In contrast, BIM protein levels were down-regulated and BCL-X<sub>L</sub> protein levels were up-regulated in RAS-activated cells (Fig. 1c). Intracellular immunostaining of BCL-X<sub>L</sub> in MCF10A KRAS<sup>V12</sup> cells showed that these cells express BCL-X<sub>L</sub> levels according to a lognormal distribution and lack an obvious subpopulation of high BCL-X<sub>L</sub> expressing cells (Fig. 1d top). However, double immunostaining of BCL-X<sub>L</sub> and CD44 revealed that cells expressing the highest levels of BCL-X<sub>L</sub> encompass subpopulations with the highest expression of CD44 (Fig. 1d bottom). We suspected from this repartition that BCL-X<sub>L</sub> might impact on the CIC phenotype and we investigated this further.

Downregulation of BCL-X<sub>L</sub> by a lentivirus based sh-RNA approach (sh-BCL-X<sub>L</sub>) had no impact on the viability of the bulk MCF10A KRAS<sup>V12</sup> population (Supplementary Fig. 2a, b). We did not detect any effect of BCL-X<sub>L</sub> knockdown on the overall doubling time of the population either (Supplementary Fig. 2c). In contrast, sh-BCL-X<sub>L</sub> diminished the percentage of mammosphere-forming cells (MFC) as strongly as sh-RNA knockdown of IL-6, a cytokine which plays a role in CIC maintenance. Sh-RNA mediated BAX knockdown was used as a control for a possible impact of the RNAi machinery on MFC and we found it had no effect (Fig. 2a). To confirm that the effects of the three sh-BCL-X<sub>L</sub> used are on-target effects, we treated with one given sh-BCL-X<sub>L</sub> KRAS<sup>V12</sup>-transformed cells after their infection with a lentivirus encoding for a sh-RNA resistant variant of BCL-X<sub>L</sub>cDNA. The resulting cells, in contrast to control cells, did not decrease their amount of mammosphere forming capacity (Supplementary Fig. 2d).

In an additional independent approach BCL2L11 (BCL-X<sub>L</sub> gene) was knocked out using CRISPR/Cas9 in KRAS<sup>V12</sup> cells. Knock out cells showed a decrease in MFC compared to control cells (Supplementary Fig. 2e). Importantly, we confirmed the involvement of BCL-X<sub>L</sub> in mammosphere formation in second-generation assays in KRAS<sup>V12</sup> cells as well as in mammosphere formation in the KRAS wild type human breast cancer cell line MDA-MB-468 (Supplementary Fig. 2f, g). A role for BCL-X<sub>L</sub> in self-renewal was also found in a non-transformed context, since
**Fig. 1** Oncogenic RAS induces BCL-XL expression correlated with enhanced CD44 expression.  

**a** qPCR analysis of BCL2 family member mRNA expression in MCF10A Lxsn and KRASV12 cell lines grown in adherent conditions. Mean and SEM of 3 independent experiments are represented as relative quantity of mRNA normalised to the mean of RPLP0, RPS18 and B2M relative expression (two-tailed unpaired t-test). **b** Western blot showing KRAS, BCL-XL, MCL-1 and BIM expression in MCF10A Lxsn and KRASV12 cell lines. **c** qPCR analysis of BCL2L1 and BCL2L11 mRNA expressed in MCF10A KRASV12 cell line treated with 10 μM of RAF inhibitor (L779450). Mean and SEM of three independent experiments are represented as relative quantity of mRNA normalised to the mean of RPLP0, RPS18 and B2M relative expression (two-tailed unpaired t-test). **d** Flow cytometry analysis of co-staining for intracellular BCL-XL and cell surface CD44. Expression of CD44 (middle and bottom) is shown in populations of increasing levels of BCL-XL as evaluated in top panel (grey, EGF treated Lxsn cells, blue, KRASV12 cell). Stainings with control isotypes are shown in green. Data from one representative experiment are shown.
EGF treatment of MCF10A Lxsn induced BCL-XL expression (Fig. 2b) and increased the percentage of MFC in a BCL-XL dependent manner (Supplementary Fig. 1b and 2c). Moreover, overexpression of BCL-XL promoted EGF-induced sphere forming in MCF-7 cell line (see below). Altogether, these results highlight a role for BCL-XL in self-renewal in oncogenic and non-oncogenic RAS activated models.

We confirmed a role for BCL-XL in CIC induction by KRASV12 in vivo by evaluating the ability of a minimal number of BCL-XL-depleted MCF10A KRASV12 cells to seed new tumours in immunodefi cient mice (Fig. 2d left). BCL-XL-deicient cells initiated tumours that seemed to grow with similar rates than control cells but with a signifi cant delay (Fig. 2d right). This is consistent with an effect of BCL-XL on the initial number of tumour seeding cells and not on tumour progression per se.

We further examined if the canonic anti-apoptotic function of BCL-XL could explain its impact on CIC maintenance. No cell death rates were detected in KRASV12-induced mammospheres following sh-BCL-XL (Supplementary Fig. 3a). Caspase inhibition did not rescue mammosphere formation in these conditions and no effect on mammosphere formation was detected upon BH3 mimetic (ABT-737) treatment (Supplementary Fig. 3b, c). Altogether, these results indicate that the effect of BCL-XL on CIC representation does not ensue from an impact on CIC viability and does not rely on its canonical anti-apoptotic activity (See Supplementary Note 2 for more details). Instead, BCL-XL appears to directly regulate some features of CICs. Consistent with this, we found that sh-BCL-XL decreased the representation of CD44high cells in the MCF10A KRASV12 population (Fig. 2e).

BCL-XL supports RAS activation to induce HMGA2 and FOSL1 expression. We performed iTRAQ labelling and quantitative mass spectrometric analysis of protein lysates from EGF-treated MCF10A Lxsn cells and MCF10A KRASV12 cells depleted or not in BCL-XL17. We used four different labelling reagents to compare protein expressions between each of the different cell contexts (Supplementary Fig. 4. Please also see Supplementary Methods for more details). We thus identifi ed proteins whose expression was induced by RAS activation or affected by BCL-XL in conditions promoting MFC (i.e. EGF-treated MCF10A and MCF10A KRASV12 cells). Proteins were called as “BCL-XL-dependent” when their fold change log2 expression was higher than 0.28 or when their fold change log2 expression was lower than -0.18 following BCL-XL knockdown in MCF10A KRASV12 cells or in EGF-treated MCF10A Lxsn cells (bottom 30% representing respectively 668 and 612 proteins). Proteins were called as “RAS-induced” when their fold change log2 expression was higher than 0.28 in MCF10A KRASV12 cells compared to EGF-treated Lxsn (top 30% representing 648 proteins). By this approach, we identifi ed 118 proteins whose expression was “RAS-induced” and “BCL-XL dependent” in both cell lines (Table 1).

We identifi ed CD44 expression as RAS-induced BCL-XL-dependent (in line with the result shown in Fig. 2e) and KRAS...
BCL-X<sub>L</sub> contributes to MAPK activation to induce HMGA2 expression. a Venn diagram of repartition of protein expression variations in iTraq experiment. Applied cut-offs on log2 Fold Change are mentioned for each condition. RAS-induced proteins are those for which the log2 Fold Change is higher than 0.28 in MCF10A KRAS<sup>V12</sup> sh-Ctl compared to MCF10A Lxsn sh-Ctl. BCL-X<sub>L</sub>-dpdt proteins are those for which the log2 fold change is lower than −0.18 in sh-BCL-X<sub>L</sub> compared to sh-Ctl in either EGF-treated Lxsn cells (right) or MCF10A KRAS<sup>V12</sup> cells (left). b Western blot showing KRAS, BCL-X<sub>L</sub> and/or p-ERK expressions in b MCF10A Lxsn and KRAS<sup>V12</sup> cells, c MCF10A KRAS<sup>V12</sup> cells 72 h after sh-BCL-X<sub>L</sub> and d MCF10A Lxsn cells 72 h after sh-BCL-X<sub>L</sub> (in presence of 20 ng ml<sup>−1</sup> EGF in MCF10A Lxsn media). e Correlations between BCL-X<sub>L</sub> protein expression and KRAS protein expression (left) or p338RAF protein expression (right) in basal subtype tumour samples. Quantified expression of BCL-X<sub>L</sub>, KRAS and p338RAF from RPPA data were examined for correlation using Pearson’s (Pear.) analysis. The results shown here are based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/. f Box & Whiskers representation of KRAS<sup>V12</sup> vs. Lxsn log2 Fold Change protein expression (Tukey representation, unpaired t-test with equal SD). Black box represents log2FC for all proteins, dark blue box represents KRAS vs. Lxsn log2FC of BCL-X<sub>L</sub>-dependent proteins (as defined above), light blue box represents KRAS vs. Lxsn log2FC of BCL-X<sub>L</sub>-independent proteins (with sh-BCL-X<sub>L</sub> vs. sh-Ctl log2FC <−0.18 or >0.18 in KRAS<sup>V12</sup> background). g qPCR analysis of HMGA2 mRNA in MCF10A Lxsn and KRAS<sup>V12</sup> cell lines infected with sh-BCL-X<sub>L</sub> during 72 h (in presence of 20 ng ml<sup>−1</sup> EGF in MCF10A Lxsn media). Mean and SEM of 3 independent experiments are represented as relative quantity of mRNA normalised to the mean of RPLPO, RPS18, and GAPDH relative expression (two-tailed unpaired t-test). Western blot analysis showing HMGA2 expression in MCF10A Lxsn cell line infected with sh-BCL-X<sub>L</sub> during 72 h.
Table 1 BCL-XL-dependent RAS-induced proteins

| BCL-XL | RAS-induced Proteins |
|--------|----------------------|
| ABC1   | Dynacin                |
| AC01   | MAP1B                 |
| ACT1A  | EIF2AK2               |
| ACT3R  | ENAH                  |
| AHS1A  | ERGIC1                |
| AP2A2  | ESYT1                 |
| AP251  | ETPF                  |
| ARL6IP5| FKBP3                 |
| ARPC5  | FNMI2                 |
| ATP1B1 | GABARAP2L             |
| ATP6V1A| GALNT2                |
| B4AGLT1| GFM1                  |
| BCL2L13| GFPT2                |
| BSG    | GLUD1                |
| C1QBP  | GPA1A                 |
| CALR   | HMGMA2                |
| CAPG   | HNRNPUL2              |
| CAPRIN1| HYO1UI                |
| CD44   | IID3B                 |
| CFL2   | ID1I                  |
| CKAP4  | IKBKGI                |
| CNPY2  | IMPDH1                |
| COPA   | IGPAP3                |
| CRIP2  | ITGA3                 |
| CS     | ITGA5                 |
| CSTB   | KLCl                  |
| CYBS3  | KRAS                  |
| DDO5T  | KYNJU                |
| DECR1  | LAMBI                |
| DUSP23 | LMN2                 |

List of proteins found in the MS analysis whose are common elements of “RAS-induced,” “BCL-XL-dep (KRASV12)” and “BCL-XL-dep (Lxsn)” illustrated in and described in Fig. 3a. RAS-induced proteins are those for which the log2 Fold Change is higher than 0.28 in MCF10A KRASV12 cells and inhibited in both models by low doses of L779450 (Fig. 4a). In contrast, expressions of BCL2L1 (BCL-XL), BCL2L11 (BIM) and CCND1 (a cell cycle regulator induced by the numerous transcription factors that RAS activates) were only affected at higher concentrations. Similar results were obtained using a concentration range of the MEK inhibitor U0126 (Fig. 6a) as well as in EGF-treated MCF10A Lxsn cells (Fig. 4b and Supplementary Fig. 6b). We reasoned that, reciprocally, the expression of genes that are highly sensitive to inhibition of RAS signalling should show BCL-XL dependency. Expression of FOSL1, which encodes for a transcription factor that contributes to breast cell maintenance and self-renewal, is highly sensitive to changes in MAPK/ERK signalling. Consistently, its expression was higher in MCF10A KRASV12 cells than in EGF-treated MCF10A Lxsn cells and inhibited in both models by low doses of L779450 (Fig. 4a) and U0126 (Supplementary Fig. 6b). In both models sh-BCL-XL decreased FOSL1 expression even if it had no significant effect on CCND1 expression (Fig. 4c). This argues that BCL-XL is necessary for RAS-induced expression of self-renewal regulators (HMG2, FOSL1) because it supports a fully active signalling pathway downstream of stabilized RAS.

BCL-XL interacts with KRAS to favour downstream signalling. BCL-2 homologues exert their biological functions by modulating the activity of numerous binding partners. We sought for protein interactants of BCL-XL in MCF10A KRASV12 cells by immunoprecipitation of BCL-XL followed by mass spectrometric analysis and identified KRAS as a putative binding partner for BCL-XL (Supplementary Data 2). Interactions between KRAS and BCL-XL were confirmed by co-immunoprecipitation assays from MCF10A KRASV12 lysates and by pull down assays with recombinant BCL-XL and KRAS (Fig. 5a). To further attest that BCL-XL/KRAS interactions occur in a whole live cell context, and to ensure that they were not artificially favored by detergents used in the above assays, we performed BioLuminescence Energy Transfer (BRET) experiments (See Supplementary Note 3 for more details). Saturable BRET signals were observed between increasing levels of YFP-fused to the N-terminal end of KRAS and with concentrations of the RAF kinase inhibitor L779450 (Fig. 5b). In contrast, BCL-XL induced BRET signals were not affected by a single mutation in BCL-XL that affects its BH3 binding (the G138A substitution, Fig. 5c) nor by treatment with BH3 mimetics ABT-737 or WEHI-539 which inhibited BRET signals between BCL-XL and the BH3-only protein tBID (Supplementary Fig. 7). This indicates that the BCL-XL/BH3 binding interface, on which relies its canonic anti-

as bafilomycin A1 and chloroquin, but not MG132, enhanced KRAS proteins levels in BCL-XL knock out cells (Supplementary Fig. 5d). Importantly, the effect of BCL-XL on KRAS expression levels coincides with an effect on downstream signalling, as BCL-XL depletion led to decreased phosphorylation of ERK (p-ERK) in MCF10A KRASV12 cells and inhibited in both models by low doses of L779450 (Fig. 4a). In contrast, expressions of BCL2L1 (BCL-XL), BCL2L11 (BIM) and CCND1 (a cell cycle regulator induced by the numerous transcription factors that RAS activates) were only affected at higher concentrations. Similar results were obtained using a concentration range of the MEK inhibitor U0126 (Fig. 6a) as well as in EGF-treated MCF10A Lxsn cells (Fig. 4b and Supplementary Fig. 6b). We reasoned that, reciprocally, the expression of genes that are highly sensitive to inhibition of RAS signalling should show BCL-XL dependency. Expression of FOSL1, which encodes for a transcription factor that contributes to breast cell maintenance and self-renewal, is highly sensitive to changes in MAPK/ERK signalling. Consistently, its expression was higher in MCF10A KRASV12 cells than in EGF-treated MCF10A Lxsn cells and inhibited in both models by low doses of L779450 (Fig. 4a) and U0126 (Supplementary Fig. 6b). In both models sh-BCL-XL decreased FOSL1 expression even if it had no significant effect on CCND1 expression (Fig. 4c). This argues that BCL-XL is necessary for RAS-induced expression of self-renewal regulators (HMG2, FOSL1) because it supports a fully active signalling pathway downstream of stabilized RAS.

BCL-XL interacts with KRAS to favour downstream signalling. BCL-2 homologues exert their biological functions by modulating the activity of numerous binding partners. We sought for protein interactants of BCL-XL in MCF10A KRASV12 cells by immunoprecipitation of BCL-XL followed by mass spectrometric analysis and identified KRAS as a putative binding partner for BCL-XL (Supplementary Data 2). Interactions between KRAS and BCL-XL were confirmed by co-immunoprecipitation assays from MCF10A KRASV12 lysates and by pull down assays with recombinant BCL-XL and KRAS (Fig. 5a). To further attest that BCL-XL/KRAS interactions occur in a whole live cell context, and to ensure that they were not artificially favored by detergents used in the above assays, we performed BioLuminescence Energy Transfer (BRET) experiments (See Supplementary Note 3 for more details). Saturable BRET signals were observed between increasing levels of YFP-fused to the N-terminal end of KRAS and with concentrations of the RAF kinase inhibitor L779450 (Fig. 5b). In contrast, BCL-XL induced BRET signals were not affected by a single mutation in BCL-XL that affects its BH3 binding (the G138A substitution, Fig. 5c) nor by treatment with BH3 mimetics ABT-737 or WEHI-539 which inhibited BRET signals between BCL-XL and the BH3-only protein tBID (Supplementary Fig. 7). This indicates that the BCL-XL/BH3 binding interface, on which relies its canonic anti-

as bafilomycin A1 and chloroquin, but not MG132, enhanced KRAS proteins levels in BCL-XL knock out cells (Supplementary Fig. 5d). Importantly, the effect of BCL-XL on KRAS expression levels coincides with an effect on downstream signalling, as BCL-XL depletion led to decreased phosphorylation of ERK (p-ERK) in MCF10A KRASV12 cells and inhibited in both models by low doses of L779450 (Fig. 4a). In contrast, expressions of BCL2L1 (BCL-XL), BCL2L11 (BIM) and CCND1 (a cell cycle regulator induced by the numerous transcription factors that RAS activates) were only affected at higher concentrations. Similar results were obtained using a concentration range of the MEK inhibitor U0126 (Fig. 6a) as well as in EGF-treated MCF10A Lxsn cells (Fig. 4b and Supplementary Fig. 6b). We reasoned that, reciprocally, the expression of genes that are highly sensitive to inhibition of RAS signalling should show BCL-XL dependency. Expression of FOSL1, which encodes for a transcription factor that contributes to breast cell maintenance and self-renewal, is highly sensitive to changes in MAPK/ERK signalling. Consistently, its expression was higher in MCF10A KRASV12 cells than in EGF-treated MCF10A Lxsn cells and inhibited in both models by low doses of L779450 (Fig. 4a) and U0126 (Supplementary Fig. 6b). In both models sh-BCL-XL decreased FOSL1 expression even if it had no significant effect on CCND1 expression (Fig. 4c). This argues that BCL-XL is necessary for RAS-induced expression of self-renewal regulators (HMG2, FOSL1) because it supports a fully active signalling pathway downstream of stabilized RAS.

BCL-XL interacts with KRAS to favour downstream signalling. BCL-2 homologues exert their biological functions by modulating the activity of numerous binding partners. We sought for protein interactants of BCL-XL in MCF10A KRASV12 cells by immunoprecipitation of BCL-XL followed by mass spectrometric analysis and identified KRAS as a putative binding partner for BCL-XL (Supplementary Data 2). Interactions between KRAS and BCL-XL were confirmed by co-immunoprecipitation assays from MCF10A KRASV12 lysates and by pull down assays with recombinant BCL-XL and KRAS (Fig. 5a). To further attest that BCL-XL/KRAS interactions occur in a whole live cell context, and to ensure that they were not artificially favored by detergents used in the above assays, we performed BioLuminescence Energy Transfer (BRET) experiments (See Supplementary Note 3 for more details). Saturable BRET signals were observed between increasing levels of YFP-fused to the N-terminal end of wild type BCL-XL and R-Luc fused to the N-terminal end of KRAS. This was observed regardless of KRAS mutational status and thus GTP-binding state (Fig. 5b). BRET signals between BCL-XL and KRAS were neither inhibited by a single mutation in BCL-XL that affects its BH3 binding (the G138A substitution, Fig. 5c) nor by treatment with BH3 mimetics ABT-737 or WEHI-539 which inhibited BRET signals between BCL-XL and the BH3-only protein tBID (Supplementary Fig. 7). This indicates that the BCL-XL/BH3 binding interface, on which relies its canonic anti-
apoptotic function, is not directly involved. In contrast, deletion in the BH4 domain that plays a critical role in BCL-XL interactions with numerous partners outside of the BCL-2 family, significantly diminished BRET signals (Fig. 5c).

To confirm that BH4-dependent interactions between KRAS and BCL-XL account for its effect on RAS signalling, we used a BRET-based RAS activity sensor in epithelial human breast cancer MCF-7 cells stably overexpressing equivalent levels of wild type or BH4-deleted BCL-XL. This allowed us to monitor the influence of BCL-XL and its BH domain on RAS activation kinetics following EGF addition. Overexpression of wild type BCL-XL enhanced the early response to EGF and significantly prolonged it but the BH4-deleted RAS binding deficient mutant failed to do so (Fig. 5d). In further support to a critical role for the BH4 domain of BCL-XL we observed that the overexpression of wild type BCL-XL but not the BH4-deleted mutant promoted EGF-induction of FOSL1 and HMGA2 in MCF-7 cells (Fig. 5e). Likewise, BH4-deleted BCL-XL was significantly less efficient than wild type BCL-XL to favour mammosphere formation by EGF-treated MCF-7 cells (Fig. 5f).

**Fig. 4** BCL-XL-dependent RAS target genes expression are sensitive to low dose of RAF inhibitor. a qPCR analysis of BCL2L1, HMGA2, FOSL1, BCL2L11 and CCND1 mRNA in MCF10A KRASV12 cells treated with increasing doses of RAF inhibitor (L779450) during 24 h. Mean and SEM of 3 independent experiments are represented as relative quantity of mRNA normalised to the mean of RPLP0, RPS18 and ACTB relative expression. Insert: western blot showing phosphorylation of ERK and total ERK levels under the same conditions. b qPCR of BCL2L1, HMGA2, FOSL1, BCL2L11 and CCND1 mRNA in MCF10A Lxsn cells grown in the presence of EGF treated with increasing doses of RAF inhibitor (L779450) during 24 h. Mean and SEM of 3 independent experiments are represented as relative quantity of mRNA normalised to the mean of RPLP0, RPS18 and GAPDH relative expression. Insert: western blot showing phosphorylation of ERK and total ERK levels under the same conditions. c qPCR of FOSL1 and CCND1 mRNA in EGF-treated MCF10A Lxsn and in MCF10A KRASV12 cell lines infected with sh-BCL-XL during 72 h. Mean and SEM of 3 independent experiments are represented as relative quantity of mRNA normalised to the mean of ACTB, HPRT1 and GAPDH relative expression (two-tailed unpaired t-test).
In some cancers, accumulation of high BCL-XL expressing cells might emerge from a negative selection induced by cell autonomous pro-apoptotic oncogenic signals such as these resulting from enhanced MYC or decreased pRB expression. The situation appears to be different in basal-like breast cancers since BCL-XL expression is neither positively correlated to MYC expression nor negatively with that of pRB (Supplementary Fig. 5k). We found, instead, that BCL-XL expression levels correlate with those of KRAS and downstream RAF activation. This hints on a link between RAS activity, which is frequently high in basal-like breast cancers (despite rare activating mutations), and BCL-XL expression whose expression is associated with therapeutic resistance in the same cancers. This correlation is mostly

**Discussion**

In some cancers, accumulation of high BCL-XL expressing cells might emerge from a negative selection induced by cell autonomous pro-apoptotic oncogenic signals such as these resulting from enhanced MYC or decreased pRB expression. The situation appears to be different in basal-like breast cancers since BCL-XL expression is neither positively correlated to MYC expression nor negatively with that of pRB (Supplementary Fig. 5k). We found, instead, that BCL-XL expression levels correlate with those of KRAS and downstream RAF activation. This hints on a link between RAS activity, which is frequently high in basal-like breast cancers (despite rare activating mutations), and BCL-XL expression whose expression is associated with therapeutic resistance in the same cancers. This correlation is mostly

**Fig. 5** BCL-XL interacts with KRAS through its BH4 domain to favour its signalling. a Left: MCF10A KRASV12 lysate was used to perform immunoprecipitation with a control or an anti-BCL-XL antibody. Western blot immunodetection was done using anti pan-RAS or anti BCL-XL antibody. Right: Pulldown assays between His-tagged KRAS and GST-tagged BCL-XL were analysed by western blot immunodetection as in immunoprecipitation assay (see Methods for more details). b, c Interaction between KRAS and BCL-XL in MCF-7 cells was assessed by BRET saturation curve assays using increasing amount of plasmid encoding YFP-BCL-XL, YFP-BCL-XL-ΔBH4, or YFP-BCL-XL-G138A and a fixed amount of plasmid encoding Rluc-KRAS. BRET ratios were obtained for every YFP- BCL-XL plasmid concentration and plotted as a function of the ratio of total acceptor fluorescence to donor luminescence. Data were fitted using a nonlinear regression equation assuming a single binding site. Data presented are representative of three independent experiments. d RAS activity following EGF stimulation. BRET-based KRAS activity sensor (see Methods for details) was used to monitor the activity of KRAS after adding 200 nM EGF to cell medium at t = 0. Measurements were done in control cells (pLVX), BCL-XL overexpressing cells (BCL-XL) and BCL-XL-ΔBH4 overexpressing cells (ΔBH4). Data presented are representative of three independent experiments. e qPCR analysis of HMGA2, FOSL1 and BCL2L1 mRNA in MCF7 control cells (pLVX), BCL-XL overexpressing cells (BCL-XL) and BCL-XL-ΔBH4 overexpressing cells (ΔBH4) after overnight starvation and treatment with 20 ng ml⁻¹ EGF for 24 h. Mean and SEM of 3 independent experiments are represented as relative quantity of mRNA normalised to the mean of RPLP0, RPS18 and ACTB relative expression (two-tailed unpaired t-test). f Percentage of sphere forming cells in bulk population of EGF-treated MCF-7 cells stably transfected with either a control plasmid (pLVX), a plasmid encoding BCL-XL, or a plasmid encoding BCL-XL-ΔBH4. Mean and SEM of 4 independent experiments are represented (two-tailed unpaired t-test)
consistent with our mechanistic studies that provide evidence for a self-amplificatory process wherein RAS activity leads to induction of BCL-XL that in turn regulates RAS protein levels and signalling. In our assays, BCL-XL induction and BIM repression were sensitive enough to high concentrations of RAF or MEK inhibitors. This indicates that RAS targeting on its own might not be always sufficient to down-regulate BCL-XL and impact on cancer cell viability. The robustness of BCL-XL expression downstream of RAS activity is in agreement with BCL-XL described as a restrain mechanism that in turn regulates RAS protein levels and signalling. It implies, moreover, that targeting this feedback itself may represent an interesting therapeutic approach impacting some critical RAS signalling outcomes.

Attempts at controlling RAS activity have so far focused on the regulation of its GTP-binding and on post-translational impacts on some critical RAS signalling outcomes. It implies, moreover, that targeting this feedback itself may represent an interesting therapeutic approach impacting some critical RAS signalling outcomes.

Table 2 Oligo sequences used for qPCR

| ACTB  | 5′-AGAAAAATCTGCAACCAACCCAC/CAGAGAGGTACAGGATAGC-3′ |
| B2M   | 5′-CTGGGCTTACTGTGTC/AATGTCGAGTTGTAACACC-3′ |
| BAK1  | 5′-GCCACCGCCAGAATGGCT/AAGGGCAGCTGAGCCGAA-3′ |
| BAX   | 5′-GCACCTCAACTGGCCCGGG/ATGACGGCCCAACGCGTC-3′ |
| BBC3  | 5′-ACCTCAAGGACATCGACA/GCACTTTAATGGGTCACTC-3′ |
| BCL2  | 5′-CTCTTTTTTGTCCGTTGG/TCTTCAAGAAGGGAGGGG-3′ |
| BCL2A1| 5′-TGGATAAGCAAAACGAGGCTGG/CTTGTGGCCACTGCTACTACCA-3′ |
| BCL2L1| 5′-TTCAGTGCACCTGACATCCA/TCCAAACAGATGATCCGCC-3′ |
| BCL2L2| 5′-GCCCTAACACTATCTCAG/TAAGGGTAACTGCTCC-3′ |
| CCND1 | 5′-GGGCAATGCCCACACTGGAC/CCAGGCAGCCCTTACAGTGC-3′ |
| CDH1  | 5′-AGAAGTGGTCCCTCGGCC/CAGTTAGATGAGGAACGTCC-3′ |
| FNI   | 5′-AGCAAGCTACATTGAAATG/CCAAAGCTGCTGTAGATTGA-3′ |
| FOSL1 | 5′-AGCGCCAGGACTGGACAAACTG/TCTTCCGAGATTGGCATATG-3′ |
| GAPDH | 5′-CAGAGGTGTCATCTCCTGC/AGTGTGCTAGTGTAGCCTTG-3′ |
| HMG2A | 5′-AGGCCACCTGAGGAAATG/CCAAAGCTGCTGTAGATTGA-3′ |
| HPRT1 | 5′-ATGGTGAATGGAAAAGGA/GATGTAGATGGGCTCATCG-3′ |
| MCL1  | 5′-TCGGTACCTCTGAGGGACGCCC/CAGCTTTTGTAGCAGCCTGCTCT-3′ |
| PMAP1P1| 5′-CTCTGAGCTGAGGTCGAGGG/CGGAAGTTAGTGTGCTC-3′ |
| RPLF0 | 5′-ACCCACCTCCTGGAGAAACT/CCTCCTGGAGATTTAGGTGT-3′ |
| RPS18 | 5′-ATCCCTGAAGATTTCCAGACA/CCTCCTGGAGATTTAGGTGT-3′ |
| SNA11 | 5′-GACCCAGTGGCTCCGACACTA/CAGAGGTTGGGGCTGTGGTA-3′ |
| VIM   | 5′-GAGAACATTCTGCGGTAGAAGC/TCTCAGGGCTATGAGTTGA-3′ |
| ZEB1  | 5′-TGGAGGAGTACGACAGAGAAGGGG/TCTCCTGGTCCTTACATGC-3′ |
| ZEB2  | 5′-CCTATCGGGCCAGAAGGGCAC/GTGCTAGCTGACATCAGCGG-3′ |

have described an enrichment in BCL-XL expression in subsets of breast cancer cells endowed with stem cell properties. If a critical role has been assigned to BCL-XL in the viability of some embryonic or cancer stem cells, we suggest here that BCL-XL can also play a direct active role in the biology of CIC through its ability to modulate RAS activity.

The non-apoptotic role for BCL-XL we describe here implies that its enhanced expression can be advantageous before impacting survival. Thus, apoptosis resistance of BCL-XL over-expressing cancer cells is not necessarily the directly selected trait. At the core of this exaptation process, we define a direct stabilising interaction between BCL-XL and KRAS regardless of its mutational status. A similar interaction was reported to allow cell survival upon accumulation of post-translationally modified KRAS at the mitochondria. The oncogenic consequences on RAS signalling were not investigated in this study. Our observations also evoke BCL-2/HRAS interactions and their reported effects on de-differentiation of luminal breast cancer cells, by mechanisms that have not been totally described yet. We provide evidence for a role of the KRAS/BCL-XL interaction in the transition towards a CIC state together with the conceptual framework and tools to unravel its molecular basis, its regulation and biological outputs in breast cancer cell populations and CIC subsets.

Targeting the anti-apoptotic function of BCL-XL to destruct chemoresistant cells remains problematic. Currently available compounds lack full efficiency in cancer cells while inducing dose-limiting thrombocytopenia. Our description of a functional role of KRAS/BCL-XL in the transition towards a CIC state together with the conceptual framework and tools to unravel its molecular basis, its regulation and biological outputs in breast cancer cell populations and CIC subsets.

Methods

Cell lines: MCF10A Lmex and KRASV12 cells were grown in DMEM-F12 (Gibco, Saint Aubin, France) supplemented with 5% Dulbecco Horse Serum (DHS) (Eurobio, Courtabeouf, France), glutamine 2 mM (Gibco), hydrocortisone 0.5 μg ml−1 (Sigma-Aldrich), cholera toxin 100 ng ml−1 (Sigma-Aldrich), insulin...
10 μg ml⁻¹ (Sigma-Alrich), HEPES 10 mM (Sigma-Aldrich) and 20 ng ml⁻¹ EGF (PeproTech). Experiments were performed using the same medium but with 2% DMSO. When indicated, 20 ng ml⁻¹ EGF was added to MCF10A Lox cell cultures. MCF-7 cells were obtained from ATCC and grown in RPMI 1640 (Gibco) supplemented with 10% Fetal Bovine Serum (FBS) (Biovia, Bousens, France) and glutamine 2 mM. Stable models of MCF-7 or MCF10KRAS⁵¹² cells expressing pLVX (empty vector), pLVX-BCL-X₁, or pLVX-BCL-X₁ΔB₄₁₄ were obtained by transfection with Lipofectamine 2000® according to the manufacturer protocol in the case of MCF-7 cell line or by lentivirus infection in the case of MCF10A cell line. Selection was performed with 500 ng ml⁻¹ of puromycin. When specified lentiviruses were used to transduce control lentivectors or sh-RNA (MOI 5). When specified we used QVD-OPH (R&D System, Minneapolis, MN, USA), ABT-737 (Sigma, St. Louis, MO, USA), L779450 (Santa Cruz Biotechnologies, Heidelberg, Germany), U0126 (Santa Cruz, sc-30), anti-BCL-XL (abcam, ab32370), anti-MCL-1 (Santa Cruz, sc-20759), and Constant amounts (50 ng per well) of plasmid expressing

Biochemical assays. Immunoprecipitation assays were performed as follows: cells were cultured in 10 cm petri dishes and were collected and washed with PBS. Cell lysis was performed using ChIP buffer (SDS: 1% EDTA: 10 mM-Tris-Hcl pH 8.1: 50 mM+ (plus a cocktail of protease and phosphatase inhibitors)), and cellular suspensions were sonicated for 15 min thrice. 10 μl of anti-BCL-X-L (Abcam) or 2 μl of anti-GFP antibody (Abcam) were used for 500 μg of cell extract to carry out immunoprecipitations that were performed as described in the PureProteome Protein G Magnetic Beads protocol (Millipore). The pulldown protocol was adapted from the HisPur™ Protein G Magnetic Beads procedure (Thermo scientific). Briefly, 1 μg of His tagged KRAS (Abcam #a96817) was mixed to 40 μl of Ni-NTA Magnetic Beads in 400 μl of binding buffer (25 mM Tris-HCl, pH 7.2, 150 mM NaCl, 5 mM MgCl₂, 1% NP-40, 200 mM Imidazol) for 60 min in an end-over-end rotator at room temperature. Beads were then collected and washed one time with binding buffer to remove unbound proteins. 0.05 μg of GST-tagged BCL-X₁ (Clinicsine #H00005988) in 400 μl of binding buffer was added to the beads and mixed overnight at 4°C. Beads were then washed thrice in wash buffer (25 mM Tris-HCl, pH 7.2, 150 mM NaCl, 5 mM MgCl₂, 1% NP-40, 200 mM Imidazol) and analysed by western blot.

Flow cytometry analysis. Cells were harvested using trypsin, gentle agitation and washed with PBS. 300,000 cells were used for each transfection. Membrane staining was performed on fresh cells using 15 ng of CD44-APC (559 942, BD Bioscience, Le Pont de Claix, France) and 2 μg each of control APC (559 745, BD Bioscience) in PBS 0.5% BSA incubated 15 min in the dark. Cells were washed in PBS 0.5%, then fixed with 2% formaldehyde during 10 min at 37°C. Cells were washed with PBS 0.5% BSA and then permeabilised with 90% cold methanol during 30 min at 4°C and then washed twice. Intracellular staining was performed with the minimal to inject in order to obtain 100% tumour uptake in less than 5 months (not shown). The presence of a visible or palpable tumour was regularly monitored and tumour volume was measured using callipers during a period of 32 weeks. The animals were euthanised when tumour volume reached 2000 mm³ or when signs of tumour necrosis were observed.

Immunochemistry. 104 patients diagnosed and treated at the ICO Cancer Center were collected between 1998–2007 among triple negative invasive breast carcinoma (ER/PRApr and Her2 negative). Representative formalin fixed tumour blocks were selected to establish tissue microarray for 88 patients. For 16 patients, full tumour blocks was used as tissue microarray was defective.

Immunolabelling technique was performed by the Benchmark XT automated tissue staining system (Ventana Medical system) on 4 μm thick blocks section. Primary antibodies used were BCL-XL (BD pharmingen, rabbit polyclonal S56361), CC1 short PHB, dilution 1/500, and p-ERK (Phospho-p44/p42 MAPK (th2/tyr 204) Cell Signaling, rabbit monoclonal, C11 standard PHB, dilution 1/400).

For each staining, the H-Score was calculated as “intensity of staining” × “% of stained cells”, where “the intensity of staining” was graded from 0 to 5 (0 none, 1 very weak, 2 weak, 3 intermediate, 4 strong and 5 very strong) and the “percentage of stained cells” estimated from number of tumours cells with cytoplasmatic and/or nuclear staining (in case of p-ERK staining) or cytoplasmatic staining (in case of BCL-XI, staining).

BRET. RLuc expression plasmids were constructed by subcloning KRAS coding sequences into the pRLuc-C2 vector (BioSignal Packard). eYFP expression plasmids were constructed by subcloning BCL-X₁ (BD pharmingen, rabbit polyclonal S56361) into the pEYFP-C1 vector (BD Biosciences). All constructs were sequenced before use. BRET saturation curves assays: at 24 h before transfection, cells were plated in 12-well plates. Cells were transfected with increasing amounts (50 to 1500 ng per well) of plasmids coding for a BRET acceptor (eYFP-BCL-X₁, pEYFP-C1, or BRET-BCL-X₁, ΔB₄₁₄) and constant amounts (50 ng per well) of plasmid expressing the BRET donor RLuc-KRAS, using Lipofectamine 2000® (Life Technologies) according to the manufacturer’s instructions. 24 h later, cells were collected and seeded in duplicates in 96-well white plates. 24 h later, cells were treated during 16 h. Prior to BRET measurement, cells were washed once with PBS. Coelenterazine H (Interchim) at 1 μM was added 20 min before measurement. The next day, cells were monitored using the lumino/flurometer Mithras LB 940 (Berthold Technologies, France), allowing for the sequential integration of luminescence with two filter settings. The emission signal values obtained at 485 nm. The BRET ratio was calculated by subtracting the BRET signal value obtained with co-expressed donor and acceptor by that obtained with the donor protein expressed alone. Data shown were
representative of at least three independent experiments. KRAS activity sensor: expression plasmid encoded eYFP-KRAS was co-transfected with donor plasmid pBluescript-Raf (500 and 300 ng respectively per well of 12 wells plates). 24 h later, cells were collected and seeded in 96-well plates and allowed to adhere for 16 h before being starved from FBS for 24 h. EGF was added to the wells just before BRET measurements begun (200 ng ml⁻¹).

Statistical analysis of TCGA data. RPPA trimmed and RPPA subtype cells datasets established in 2012-2013 were downloaded from the TCGA data portal (https://tcga-data.nci.nih.gov/docs/publications/brea_2012). Correlation plots between proteins of interest for each breast cancer subtype and Pearson coefficient calculation were achieved using R program.

Data availability. The authors declare that the main data supporting the findings of this study are available within the article and its Supplementary Information files. Extra data are available from the corresponding author upon request.

Received: 2 January 2017 Accepted: 16 August 2017
Published online: 24 October 2017

References

1. Juiu, P., Geneste, O., Gautier, F., Depil, S. & Campone, M. Decoding and predicting of receptor tyrosine kinase (RTK) activity in breast cancer: an approach for therapeutic target identification. Mol. Cancer 13, 455–465 (2013).

2. Amundson, S. A. et al. An informatics approach identifying markers of chemoresistance in breast cancer cell lines. Cancer Res. 60, 6101–6110 (2000).

3. Wei, G. et al. Chemical genomics identifies small-molecule MCL1 repressors and BCL-xL as a predictor of MCL1 dependency. Cancer Cell 21, 547–562 (2012).

4. Mason, K. D. et al. Programmed anuclear cell death delimits platelet life span. Nature 432, 307–315 (2004).

5. Pylayeva-Gupta, Y., Grabocka, E. & Bar-Sagi, D. RAS oncogenes: wearing a tumor suppressor mask. Nat. Rev. Cancer 11, 761–774 (2011).

6. Downward, J. Targeting RAS signalling pathways in cancer therapy. Nat. Rev. Cancer 3, 11–22 (2003).

7. Loboda, A. et al. A gene expression signature of RAS pathway dependence predicts response to PI3K and RAF pathway inhibitors and expands the population of RAS pathway activated tumors. BMC. Med. Genomics. 3, 26 (2010).

8. Brahm, F., de Carne Treesscon, S., Bertin-Cifo, J. & Juiu, P. Protection and serve: Bcl-2 proteins as guardians and rulers of cancer cell survival. Cell Cycle 12, 2937–2947 (2013).

9. Mani, S. A. et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133, 704–715 (2008).

10. Morel, A. P. et al. Generation of breast cancer stem cells through epithelial-mesenchymal transition. PLoS ONE 3, e2888 (2008).

11. Dean, M. Cancer stem cells: Implications for cancer causation and therapy resistance. Discov. Med. 5, 278–282 (2005).

12. Diehn, M. & Clarke, M. F. Cancer stem cells and radiotherapy: new insights into tumor radioresistance. J. Natl. Cancer Inst. 98, 1755–1757 (2006).

13. Konishi, H. et al. Knock-in of mutant K-ras in nontumorigenic human embryonic stem cells via IL6 secretion. Breast Cancer Res. 13, 846–849 (2013).

14. Albeck, J. G., Mills, G. B. & Brugge, J. S. Frequency-modulated pulses of ERK activity transmit quantitative proliferation signals. Mol. Cell 49, 249–261 (2013).

15. Tam, W. L. et al. Protein kinase C alpha is a central signaling node and therapeutic target for breast cancer stem cells. Cancer Cell 24, 347–364 (2013).

16. Corcoran, R. B. et al. Synthetic lethal interaction of combined BCL-XL and MEK inhibition promotes tumor regressions in KRAS mutant cancer models. Cancer Cell 23, 121–128 (2013).

17. Pfleger, C. M. Ubiquitin on ras: warden or partner in crime? Sci. Signal. 4, pe12 (2011).

18. Lawson, D. A. et al. Single-cell analysis reveals a stem-cell program in human metastatic breast cancer cells. Nature 526, 131–135 (2015).

19. Avery, S. et al. BCL-XL mediate the strong selective advantage of a 20q11.21 amplification commonly found in human embryonic stem cell cultures. Stem Cell Reports 1, 379–386 (2013).

20. Nguyen, H. T. et al. Gain of 20q11.21 in human embryonic stem cells improves cell survival by increased expression of Bcl-xl. Mol. Hum. Reprod. 20, 168–177 (2014).

21. Rivnaya, T. G. et al. PKC regulates a farnesyl-electrostatic switch on K-Ras that promotes its association with Bcl-XL on mitochondria and induces apoptosis. Mol. Cell 21, 481–493 (2006).

22. Wang, X. et al. ReBI NF-kappaB represses estrogen receptor alpha expression via induction of the zinc finger protein Blimp1. Mol. Cell Biol. 29, 3832–3844 (2009).

23. Pfaff, M. W. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic. Acids. Res. 29, e45 (2001).

24. Vandemesompe, J. et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome. Biol. 3, RESEARCH0034 (2002).

25. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. Nature 490, 61–70 (2012).

Acknowledgements

We thank members of the “Stress adaptation and tumor escape” laboratory for their support. We are particularly indebted to D. L. Maillet for his help and criticism. We thank Dr Y. Guillemin for his help and inspiration at the beginning of this work and P. East for proof reading this manuscript. We thank Dr R. Rimokh and Prof. G. Gillet for fruitful discussions. We thank Dr Ho Park for his generous gift of the MCFP10-A cells line and Dr G. Micheau for his gift of plVX (BCL-XL). We thank C. Guevara for her technical help in the preparation of lentivirus particles. We benefited from invaluable technical support from the Cytometry Core facility (CytoCell) of the Federative Research Structure François Bonamy (Nantes). Dr S. de Carné Trécesson was supported by Institut National du Cancer, Dr J. Pécor by Ministère de la Recherche et de l’Enseignement Supérieur et Dr A. Basserville by Fondation de France. This work was supported by Canceropole Grand Ouest (CIC project 2010–2012, MATURE project 2017–18), ARC (R15083NN), Fondation de France (2015) and INCA PLBio (R12134NN) to PJ, Pigeau Grand Ouest (R13137) to S.C.T. and P.P.J and Fondation de France (2017) to A.B.

Author contributions

S.C.T., F.S., A.B., A.-C.B., J. P., M.B., K.A.S., S.B.N., I.V., O.C., C.G., F.G., and P.P.J. conducted experiments. S.C.T., J.L., A.L., S.B.N., M.C., F.G., and P.P.J. analyzed the data. S.C.T., F.G., and P.P.J. wrote the paper. S.C.T., J.L., A.L., S.B.N., M.C., F.G., and P.P.J. designed the experiments. S.C.T., F.S., A.-C.B., K.A.S, S.B.N., I.V., O.C., C.G., F.G., and P.P.J. performed the experiments. S.C.T., F.S., A.-C.B., K.A.S, S.B.N., I.V., O.C., C.G., F.G., and P.P.J. designed the experiments. S.C.T., F.S., A.-C.B., K.A.S, S.B.N., I.V., O.C., C.G., F.G., and P.P.J. analyzed the data. S.C.T., F.S., A.B., and P.P.J. wrote the paper. S.C.T., A.B. and P.P.J. obtained funding. P.P.J. conceived the study and supervised it.

Additional information

Supplementary Information accompanies this paper at doi:10.1038/s41467-017-01079-1.

Competing interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2017