Supporting Figures for:

Gene expression profiling of a hypoxic seizure model of epilepsy suggests a role for mTOR and Wnt signaling in epileptogenesis

Joachim Theilhaber, Sanjay N. Rakhade, Judy Sudhalter, Nayantara Kothari, Peter Klein, Jack Pollard, Frances E. Jensen.
Supporting Figures Legends

(references refer to the main text)

**Figure S1**  **Mitosis gene set under baseline developmental conditions.** A. Log2-ratios of gene expression relative to the 1h time point, in each tissue separately, are displayed as a heat map, with sampling times post-P10 indicated in hours. Colors saturate for log2-ratio = ±1. Strong repression of many genes is observed, including for the genes coding for the chromosomal passenger proteins aurora kinase B (AURKB) and surviving (BIRC5), and for several centromeric proteins. B. KS plot showing the distribution of log2-ratios at t = 1 week for both tissues. P-value and enrichment are reported for hippocampus only. Hipp = hippocampus, cx = cortex. The arrows point to some of the genes referred to in the main text.

**Figure S2**  **Gene set containing sonic hedgehog (SHH) target genes under baseline developmental conditions.** A. Strong repression of most genes in the gene set is observed by t = 1 week. B. Detailed intensity profiles for CXCR4 and TOP2A. C. KS plot showing the distribution of the log2-ratios at t = 1 week for both tissues. See Figure S1 for key to figure color and scale details.

**Figure S3**  **Neural stem cell markers under baseline developmental conditions.** A. Repression of several genes in the gene set is observed by t = 1 week, including CXCR4, CD24 and nestin (NES). B. Detailed intensity profile for CD24. c) KS plot showing the distribution of the log2-ratios at t = 1 week for both tissues. See Figure S1 for key to figure color and scale details.

**Figure S4**  **Neuronal progenitor markers under baseline developmental conditions.** A. Repression of several genes in the gene set is observed by t = 1 week, including doublecortin (DCX) and OLIG2. B. KS plot showing the distribution of the log2-ratios at t = 1 week for both tissues. See Figure S1 for key to figure color and scale details.

**Figure S5**  **A gene set containing multiple neuronal markers under baseline developmental conditions.** a) Heat map of gene profiles. b) KS plot showing the distribution of the log2-ratios at t = 1 week for both tissues (P-value and enrichment here displayed for cortex). See Figure S1 for key to figure color and scale details.

**Figure S6**  **A gene set containing an empirical collection of neuronal markers under baseline developmental conditions.** The markers were obtained from the Allen Brain Atlas [20]. A. Heat map of gene profiles. B. KS plot showing the distribution of the log2-ratios at t = 1 week for both tissues. See Figure S1 for key to figure color and scale details.
Figure S7  A comprehensive collection of synaptic markers under the baseline developmental conditions. A. Heat map of gene profiles, showing the 22 genes most induced in cortex. Gene descriptions are given on the right, with functional categories color-coded as specified in the legend. B. Detailed intensity profiles for the chloride transporter KCC2. C. KS plot showing the distribution of the log2-ratios at t = 1 week for both tissues. See Figure S1 for key to figure color and scale details.

Figure S8  Astrocytic markers under the baseline developmental conditions. A. Heat map of gene profiles, showing induction of several genes, including connexin 30 (GJB6), glutamine synthesase (GLUL) and GLT-1 (SLC1A2). B. Detailed intensity profiles for GFAP, AQP9 and connexin 30. C) KS plot showing the distribution of the log2-ratios at t = 1 week for both tissues. See Figure S1 for key to figure color and scale details.

Figure S9  A gene set containing an empirical collection of oligodendrocytic markers under the baseline developmental conditions. The markers were obtained from the Allen Brain Atlas [20]. A. Heat map of gene profiles, showing very strong induction of many genes, including genes for the myelinating proteins already indicated in Figure 4 (left-hand arrows). A. KS plot showing the distribution of the log2-ratios at t = 1 week for both tissues, with very marked leading edges. See Figure S1 for key to figure color and scale details.

Figure S10  NMDA receptor components under the baseline developmental conditions. A. Heat map of gene profiles, showing induction of NR1 and NR2C and repression of NR3A and NR2D. B. Detailed log2-ratio profiles for NR1, NR2C, NR3A and NR2D in hippocampus and C, in cortex. See Figure S1 for key to figure color and scale details.

Figure S11  AMPA receptor components under the baseline developmental conditions. A. Heat map of gene profiles, showing non-significant changes in hippocampus, and repression of GLUR1 and GLUR2 in cortex. B. Detailed log2-ratio profiles for GLUR2 in hippocampus and C, for GLUR1 and GLUR2 in cortex. See Figure S1 for key to figure color and scale details.

Figure S12  GABA receptor components and glutamate decarboxylase 1 and 2 under the baseline developmental conditions. A. Heat map of gene profiles, showing generally non-significant changes. B. Detailed log2-ratio profiles for GABA_A-δ (GABRD), GABA_A-β1 (GABRB1) and KCC2 in hippocampus and C, in cortex. See Figure S1 for key to figure color and scale details.

Figure S13  Heat map of the differential response to hypoxic seizures for the genes in the In vitro response (BIC or 4AP) group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. Colors represent log2-ratios of intensities of hypoxic seizure samples to that of time-matched controls (red
= positive, green = negative values, colors saturate at ±1). Arrows point to genes referred to in the main text.

**Figure S14**  Heat map of the differential response to hypoxic seizures for the genes in the Hypoxia response group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. See Figure S13 for key to figure color and scale details.

**Figure S15**  Heat map of the differential response to hypoxic seizures for the genes in the Glutamatergic/NMDA receptor I group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. See Figure S13 for key to figure color and scale details.

**Figure S16**  Heat map of the differential response to hypoxic seizures for the genes in the Glutamatergic/NMDA receptor II group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. See Figure S13 for key to figure color and scale details.

**Figure S17**  Heat map of the differential response to hypoxic seizures for the genes in the Synaptic group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. See Figure S13 for key to figure color and scale details.

**Figure S18**  Heat map of the differential response to hypoxic seizures for the genes in the Axonal Guidance group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. See Figure S13 for key to figure color and scale details.

**Figure S19**  Heat map of the differential response to hypoxic seizures for the genes in the Autism associated group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. See Figure S13 for key to figure color and scale details.

**Figure S20**  WNT3A target genes are enriched in the hypoxic seizure response profile. Enrichment analysis with the WNT3A target gene set[27] was performed against the 12 hour profile of transcriptional response to hypoxic seizures in hippocampus. A. KS plot showing enrichment profile. B. Table of the corresponding 15 leading-edge genes. Ratios are of gene expression intensities in hypoxic seizure sample to corresponding control.

**Figure S21**  Expression ratios as a function of time for selected IGF-1/PI3K/mTOR pathway genes. The gene name is indicated at the top of each figure. Time at the bottom refers to the duration following hypoxic seizures; the left-hand panels (labeled “hyp”) display ratios of hypoxic seizure response to control; the right-hand panels (labeled “hyp + NBQX”) displays ratios of response to combined hypoxic seizures and NBQX treatment, to control. The expression ratio R = 1 (no fold change) is indicated
by the black horizontal lines. A. IRS1 displays ~ 1.6 fold induction at 12 h. B. AKT3 is very moderately but significantly induced over the time course. C and D. IGF1 and BDNF are not significantly induced.

**Figure S22**  Heat map of differential expression for the NBQX-responsive genes. The profiles of the 257 genes, each of which is differentially regulated by both hypoxic seizures (HS) relative to control, and by HS+NBQX relative to HS, are shown (these genes form a proper subset of the 1,399 HS-responsive genes displayed in Figure 6). Log2 ratios of gene expression between time-matched samples are shown for the ratio combinations indicated on top. Colors saturate for log2-ratio = ±1.

**Figure S23**  Expression ratios as a function of time for 11 Wnt genes represented on the microarray. The gene name is indicated at the top of each figure. Time at the bottom refers to the duration following hypoxic seizures; the left-hand panels (labeled “hyp”) display ratios of hypoxic seizure response to control; the right-hand panels (labeled “hyp + NBQX”) displays ratios of response to combined hypoxic seizures and NBQX treatment, to control. The expression ratio R = 1 (no fold change) is indicated by the black horizontal lines. Only WNT5A and WNT2 are significantly induced.
Figure S1

### A. Mitosis

|       | hipp | cx   |
|-------|------|------|
| BUB1B | 6    | 12   |
| CENBP | 48   | 168  |
| CENPE | 6    | 12   |
| NAB2L1| 48   | 168  |
| BUB1  | 168  |      |
| Survivin |      |      |
| Aurora kinase B |      |      |
| KIF23 |      |      |
| NEK3  |      |      |
| NEK8  |      |      |
| NAB2L2|      |      |
| CEP55 |      |      |
| PFKD5 |      |      |
| NEK4  |      |      |
| NEK5  |      |      |
| BUB3  |      |      |
| INCENP|      |      |
| NUMA1 |      |      |
| CENPF |      |      |
| CENPF |      |      |
| NEK7  |      |      |

### B. Statistics

- **P < 10^{-5}**
- **C_R = 9.3**

**Sample**

- cx, hipp 1 week

**Population**

- **logRatio**
  - 1
  - 2
Figure S2

SHH target genes

A

| Gene       | hippocampus (hipp) | cortex (cx) |
|------------|--------------------|-------------|
| CXCR4      |                    |             |
| MYCN      |                    |             |
| TOP2A     |                    |             |
| CCNA2     |                    |             |
| UBE2C     |                    |             |
| HMGB1     |                    |             |
| RACGAP1   |                    |             |
| CCNB1     |                    |             |
| MCM10     |                    |             |
| HRG       |                    |             |
| RAS       |                    |             |
| AKR1B1    |                    |             |
| KIF4A     |                    |             |
| NFIC      |                    |             |
| DCX       |                    |             |
| GAS5      |                    |             |
| TMF6      |                    |             |
| MCM3      |                    |             |
| DHMA1     |                    |             |
| TNFR1     |                    |             |
| E2F1      |                    |             |
| XPO1      |                    |             |
| CDC20     |                    |             |
| MDR2      |                    |             |
| XRCC5     |                    |             |
| MCM2      |                    |             |
| USP47     |                    |             |
| FEN1      |                    |             |
| NLR52     |                    |             |
| CCNB1     |                    |             |
| ARFGP1    |                    |             |
| RAN       |                    |             |
| NET1      |                    |             |
| MFN1      |                    |             |
| DDX15      |                   |             |
| AURK1     |                    |             |
| TOP2A     |                    |             |
| BIRC5     |                    |             |
| RANBP2    |                    |             |
| METTL5    |                    |             |
| MFN2      |                    |             |
| DDIT3     |                    |             |
| ARFGF1    |                    |             |
| ELOVL4    |                    |             |
| NT5E      |                    |             |
| FGNA      |                    |             |
| BHLHE2    |                    |             |

logTransformed values

B

CXCR4

C

TOP2A

C

SHH regulated DCP over 1 week

P < 10^-5

CR = 12.6

Sample

Population

BIRC5, hipp 1 week
Figure S3

Neural stem cells

A

|        | hipp | cx | hippoc | CXCR4 | CD24 | FUT4 | GNL3L | CDH2 | NES | HES5 | NOTCH1 | NCDF2 | CD81 | PRDM1 | THY1 | NCAM1 |
|--------|------|----|--------|-------|------|------|-------|------|-----|------|--------|-------|------|-------|------|-------|
| 6      | 12   | 48 | 168    | 6     | 12   | 48   | 168   |      |     |      |        |       |      |       |      |       |

- CXCR4: chemokine (C-X-C motif) receptor 4
- CD24: CD24 molecule
- FUT4: fucosyltransferase 4 (alpha 1-3) fucosyltransferase
- GNL3L: guanine nucleotide binding protein-like 3 (nucleolar)
- CDH2: cadherin 2 type 1 N cadherin (neuronal)
- NES: nestin
- HES5: hairy and enhancer of split 5 (Drosophila)
- NOTCH1: Notch homolog 1 translocation-associated (Drosophila)
- NCDF2: multiple coagulation factor deficiency 2
- CD81: CD81 molecule
- PRDM1: prionin 1
- THY1: Thy-1 cell surface antigen
- NCAM1: neural cell adhesion molecule 1

B

CD24

P = 1.6e-2
C_R = 8.6

C

Sample cx, hipp 1 week

Population
Figure S4

**Neuronal progenitors**

|   | hipp | cx  |
|---|------|-----|
| 6 | 12   | 48  |
| 6 | 12   | 48  |

- **DGX1**: doublecortin; Iasenophaly X-linked (doublecortin)
- **ASCL1**: achaete-scute complex homolog 1 (Drosophila)
- **OLIG2**: oligodendrocyte lineage transcription factor 2
- **NES**: nestin
- **CSPG4**: chondroitin sulfate proteoglycan 4 (melanoma-associated)
- **PDGFRα**: platelet-derived growth factor receptor alpha polypeptide
- **HES1**: hairy and enhancer of split 1 (Drosophila)
- **OLIG1**: oligodendrocyte transcription factor 1

**P = 6.7e-2**

**CR = 1.9**

B Sample

1 week

cx, hipp
**Figure S5**

### A

| Gene          | Hipp | Cx |
|---------------|------|----|
| Dcx           | 6    |    |
| Hes5          | 10   |    |
| Slc3a4        | 12   |    |
| Nta           | 168  |    |
| Trpv3         | 6    |    |
| Thr           | 12   |    |
| Il13          | 48   |    |
| Ngrt2         | 168  |    |
| Tubb3         | 6    |    |
| Ox42          | 12   |    |
| Nurod1        | 168  |    |
| Ntrk3         | 48   |    |
| Ascl1         |      |    |
| Elavl3        | 12   |    |
| Elavl4        | 48   |    |
| Gap43         |      |    |
| Mtap          | 168  |    |
| Elavl2        | 6    |    |
| Pax5          | 12   |    |
| Obh           |      |    |
| Tubam         | 48   |    |
| Slc3a3        |      |    |
| Neurod3       | 168  |    |
| Gap2          | 12   |    |
| Slc25a5       | 48   |    |
| Lkk1          | 168  |    |
| Syt1          | 6    |    |
| Nef3          | 12   |    |
| Sip1          | 168  |    |
| Slc3a1        | 12   |    |
| Syn1          | 48   |    |
| Enc2          | 168  |    |
| Ntrk1         | 6    |    |
| Slc25a2       | 12   |    |
| Nppa          | 48   |    |
| Gap1          | 168  |    |
| Ntrk2         | 6    |    |
| Nefl          | 12   |    |
| Chba1         | 48   |    |
| Ca5le         | 168  |    |
| Btg2          |      |    |
| Aeg2a2        | 168  |    |
| Aavl2         | 6    |    |
| Ppp1r1b       |      |    |
| Ncam1         | 12   |    |

*Note: Gene expressions are indicated by color, with green indicating lower expression and red indicating higher expression.*

### B

- **P = 2.0e-2**
- **C_L = 1.9**

**Sample**

- cx, hipp

**Population**

- 1 week
Figure S6

Neuronal (ABA)

A

| Sample | cx | hipp | 6 12 48 168 |
|--------|----|------|-------------|
| ADD2   | A  | A    | A           |
| GI1A2  | A  | A    | A           |
| SMARTD2| A  | A    | A           |
| ALCAM  | A  | A    | A           |
| CAMK2B | A  | A    | A           |
| NDST1  | A  | A    | A           |
| MYOS5 | A  | A    | A           |
| GI1A4  | A  | A    | A           |
| OXLM2  | A  | A    | A           |
| UCHL1  | A  | A    | A           |
| RTN1   | A  | A    | A           |
| DPPY-E | A  | A    | A           |
| YWHAS5| A  | A    | A           |
| KFC2   | A  | A    | A           |
| LHCN5 | A  | A    | A           |
| SYNGR3| A  | A    | A           |
| SNAP25 | A  | A    | A           |
| GRINZB | A  | A    | A           |
| SYT1   | A  | A    | A           |
| PRKAR1B| A  | A    | A           |
| SLC22A17| A | A    | A           |
| RYR2   | A  | A    | A           |
| GPR162 | A  | A    | A           |
| ORAL3  | A  | A    | A           |
| LRP11  | A  | A    | A           |
| MAP2C1 | A  | A    | A           |
| SH3GL2 | A  | A    | A           |
| SYF    | A  | A    | A           |
| NDRG4  | A  | A    | A           |
| FBXW5  | A  | A    | A           |
| CHST1  | A  | A    | A           |
| ATP5E1 | A  | A    | A           |
| LHCN9  | A  | A    | A           |
| CLSTN3 | A  | A    | A           |
| BSL2   | A  | A    | A           |
| NETFL | A  | A    | A           |
| GPLC1  | A  | A    | A           |
| TUBA4  | A  | A    | A           |
| CYH2   | A  | A    | A           |
| EGF1H  | A  | A    | A           |
| PACS1H | A  | A    | A           |
| CHN1   | A  | A    | A           |
| CX3CL1 | A  | A    | A           |
| P2RY5N | A  | A    | A           |
| FAIM2  | A  | A    | A           |
| CAMK2B | A  | A    | A           |

B

P < 10^-5

CL = 2.7
Figure S8

A) Astrocytic

B) Genes and Fold Changes

C) Statistical Analysis

Population vs. Sample
Figure S9

A  Oligodendrocyte (ABA)

B  P < 10^{-5}

C_L = 16.5

Sample  cx, hipp 1 week

Population

logRatio

6 12 48 168 6 12 48 168
Figure S10

A Glutamatergic : NMDAR

B

C

log2Ratio

Time post P10 (h)

6 12 48 168

NR2C NR1

NR2D NR3A

NR2C NR1

NR2D NR3A

NR2C NR1

NR2D NR3A
Figure S11

A. Glutamatergic : AMPAR

B. Time post P10 (h)

C. Time post P10 (h)
Figure S12

A

GABAergic

B

hipp

log2Ratio

Time post P10 (h)

GABRD

GABRB1

KCC2

GAD

GAD65

GAD67

GABRG2

GABRD

GABRB1

GABRA1

GABRA4

GABRA3

GABRIR3

GAD1

GABA

SLC12A5

GABRD

GABRB1

KCC2

log2Ratio

Time post P10 (h)

C

cx

GAD

GAD65

GAD67

GABRG2

GABRD

GABRB1

GABRA1

GABRA4

GABRA3

GABRIR3

GAD1

GABA

SLC12A5

GABRD

GABRB1

KCC2

log2Ratio

Time post P10 (h)
Figure S14

Hypoxia response

Time post HS (h) | Hipp | CX
---|---|---
1 | 6 | 12 | 48 | 168

- CHRNA4- | Cholinergic receptor, nicotinic alpha 4
- EP300- | E14 binding protein p300
- CHRNA7- | Cholinergic receptor, nicotinic alpha 7
- NF1- | Neurofibrin 1 (neurofibromatosis, von Recklinghausen disease)
- VEGFA- | Vascular endothelial growth factor A
- CREBBP- | CREB binding protein (Rubinstein-Taybi syndrome)
Figure S17

Synaptic activity over time post HS (h)

| Time post HS (h) | hipp | cx |
|------------------|------|----|
| 1                |      |    |
| 6                |      |    |
| 12               |      |    |
| 48               |      |    |
| 168              |      |    |

Genes and their expression levels are color-coded based on synaptic activity.
**Figure S18**

**Axonal guidance**

| Time post HS (h) | hippocampus (hipp) | cortex (cx) |
|------------------|--------------------|-------------|
| 1                | EFH2               | EFH receptor B2 |
| 6                | EFHA3              | EFH receptor A3 |
| 12               | SLIT2              | slit homolog 2 (Drosophila) |
| 48               | MET                | met proto-oncoprotein tyrosine kinase receptor |
| 168              | OMG                | oligodendrocyte myelin glycoprotein |
| 1                | ROD01              | roundabout axon guidance receptor homolog 1 (Drosophila) |
| 6                | NRP1               | neuropilin 1 |
| 12               | LRRC4C             | leucine rich repeat containing 4C |
| 48               | SEMA3A             | sema domain immunoglobulin domain (Ig) short basic domain 3A |
| 168              | EFHA7              | EFH receptor A7 |
|                  | SEMA6A             | sema domain transmembrane domain (TM) and cytoplasmic domain 6A |

**Legend:**

- Green: Low expression
- Red: High expression
- Black: No expression
Figure S19

| Time post HS (h) | hippocampus (hipp) | cortex (cx) |
|------------------|--------------------|-------------|
| 1                | 6                  | 12          |
| 48               |                    | 168         |
| 1                | 6                  | 48          |
| 12               |                    | 48          |
| 168              |                    | 168         |

- SLC6A4
- CACNA1C
- MET
- WNT2
- CACNA1D
- TSC1
- SYNGAP1
- NR1
- MAOA
- GABRA
- NLGN3

The figure shows a heat map of autism-associated genes over time post HS (h). The x-axis represents different times (1, 6, 12, 48, 168 hours), and the y-axis represents genes like SLC6A4, CACNA1C, MET, WNT2, CACNA1D, TSC1, SYNGAP1, NR1, MAOA, GABRA, and NLGN3. The colors indicate expression levels with green being lower and red being higher.
Figure S20

| #Gene | Description                                      | Ratio | P-value |
|-------|--------------------------------------------------|-------|---------|
| KLF5  | Kruppel-like factor 5 (intestinal)               | 2.282 | 0.0446  |
| ENPP2 | Ectonucleotide pyrophosphatase/phosphodiesterase 2 | 2.158 | 0.203   |
| CHRNA1| Cholinergic receptor nicotinic alpha 1 (muscle)   | 1.895 | 0.00207 |
| RND3  | Rho family GTPase 3                              | 1.888 | 0.00237 |
| IRS1  | Insulin receptor substrate 1                     | 1.785 | 0.0034  |
| TCF4  | Transcription factor 4                           | 1.579 | 0.0061  |
| AXN2  | Axin 2 (conductin axil)                          | 1.482 | 0.00693 |
| AHR   | Aryl hydrocarbon receptor                        | 1.396 | 0.248   |
| TGFBR2| Transforming growth factor beta receptor II (70/80kDa) | 1.356 | 0.168   |
| TEC   | Tec protein tyrosine kinase                      | 1.322 | 0.147   |
| CTGF  | Connective tissue growth factor                  | 1.253 | 0.227   |
| CCND1 | Cyclin D1                                        | 1.238 | 0.091   |
| SPRY1 | Sprouty homolog 1 antagonist of FGF signaling (Dros) | 1.180 | 0.277   |
| IGF1  | Insulin-like growth factor 1 (somatomedin C)     | 1.133 | 0.0652  |
| TGFBR2| Transforming growth factor beta 2                 | 1.115 | 0.178   |
Figure S21
Figure S22

| tissue ratio | hippocampus | cortex | hippocampus | cortex |
|--------------|-------------|--------|-------------|--------|
| time (h)     | HS CON 1 16 48 168 | HS+NBQX CON 6 12 48 168 | HS CON 1 16 48 168 | HS+NBQX CON 6 12 48 168 |
| HS           | LOC83891979 | LOC83891979 | LOC83891979 | LOC83891979 |
| HS+NBQX      | LOC83891979 | LOC83891979 | LOC83891979 | LOC83891979 |
| HS           | LOC83891979 | LOC83891979 | LOC83891979 | LOC83891979 |
| HS+NBQX      | LOC83891979 | LOC83891979 | LOC83891979 | LOC83891979 |
| HS           | LOC83891979 | LOC83891979 | LOC83891979 | LOC83891979 |
| HS+NBQX      | LOC83891979 | LOC83891979 | LOC83891979 | LOC83891979 |
| HS           | LOC83891979 | LOC83891979 | LOC83891979 | LOC83891979 |
| HS+NBQX      | LOC83891979 | LOC83891979 | LOC83891979 | LOC83891979 |
| HS           | LOC83891979 | LOC83891979 | LOC83891979 | LOC83891979 |
| HS+NBQX      | LOC83891979 | LOC83891979 | LOC83891979 | LOC83891979 |
| HS           | LOC83891979 | LOC83891979 | LOC83891979 | LOC83891979 |
| HS+NBQX      | LOC83891979 | LOC83891979 | LOC83891979 | LOC83891979 |

HS: high temperature; CON: control; NBQX: nabilox. Cytoscape diagram showing the expression of 257 genes under different conditions.
Figure S23