SUPPLEMENTAL INFORMATION

LLY-507, a Cell-Active, Potent and Selective Inhibitor of Protein Lysine Methyltransferase SMYD2

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| Protein      | Substrate (µM) | pH | Buffer    | DTT (mM) | TCEP (mM) | Triton-X100 (%) | Enzyme (nM) |
|--------------|---------------|----|-----------|----------|-----------|-----------------|-------------|
| PRMT1        | 0.13          | 4.6| Tris-HCl  | 5        | 0         | 0.01            | 15          |
| PRMT3        | 0.57          | 28.3| Tris-HCl  | 5        | 0         | 0.01            | 20          |
| PRMT6        | 0.6           | 2.3| Tris-HCl  | 5        | 0         | 0.01            | 50          |
| PRMT8        | 0.7           | 2.2| Tris-HCl  | 5        | 0         | 0.01            | 20          |
| G9a          | 0.8           | 8  | potassium phosphate | 0       | 0         | 0.01            | 5           |
| EHMT1 (GLP)  | 0.6           | 8  | potassium phosphate | 0       | 0         | 0.01            | 5           |
| SUV39H2      | 0.5           | 2.6| potassium phosphate | 0       | 0         | 0.01            | 10          |
| SETDB1       | 1.7           | 15 | Tris-HCl  | 5        | 0         | 0.01            | 10          |
| SETD2        | 7.5           | 1  | Tris-HCl  | 5        | 0         | 0.01            | 500         |
| SETD7        | 2             | 2  | Tris-HCl  | 5        | 0         | 0.01            | 20          |
| SETD8        | 40            | 60 | Tris-HCl  | 0        | 2         | 0.01            | 50          |
| DOT1L        | 1             | 1  | Tris-HCl  | 5        | 0         | 0.01            | 10          |
| SUV420H1     | 2.8           | 12.5| Tris-HCl  | 5        | 0         | 0.01            | 100         |
| SUV420H2     | 0.9           | 9  | Tris-HCl  | 5        | 0         | 0.01            | 500         |
| DNMT1        | 0.6           | 2  | Tris-HCl  | 5        | 0         | 0.01            | 100         |
| SMYD2        | 3             | 0.5| Tris-HCl  | 5        | 0         | 0.01            | 30          |
| PRDM9        | 3             | 140| Tris-HCl  | 5        | 0         | 0.01            | 1           |
| SMYD3        | 1             | 10 | Tris-HCl  | 5        | 0         | 0.01            | 500         |
| NSD1         | 0.2           | 2  | Tris-HCl  | 0        | 2         | 0.01            | 20          |
| NSD2         | 0.4           | 3.6| Tris-HCl  | 0        | 2         | 0.01            | 50          |
| NSD3         | 0.3           | 3.7| Tris-HCl  | 0        | 2         | 0.01            | 50          |
| PRMT5-MEP50 Complex | PRMT5 MEP50 | 0.12 | 2 | 8 | Tris-HCl | 5 | 0 | 0.01 | 15 |
| MLL1 Complex | MLL1 ASH2L RBBP5 WDR5 | 2 | 2 | 8 | Tris-HCl | 5 | 0 | 0.01 | 20 |
| MLL3 Complex | MLL3 ASH2L RBBP5 WDR5 | 12 | 55 | 9 | Tris-HCl | 5 | 0 | 0.01 | 100 |
| EZH1 Complex | EED EZH1 SUZ12 RBBP4 AEBP2 | 1 | 4 | 8 | Tris-HCl | 5 | 0 | 0.01 | 10 |
| EZH2 Complex | EED EZH2 SUZ12 | 1 | 2 | 8 | Tris-HCl | 5 | 0 | 0.01 | 20 |
SUPPLEMENTAL TABLE 2: Effect of LLY-507 on the activity of 454 human kinases. LLY-507 was profiled for kinase inhibitory activity using the DiscoverX KINOMEscan® Assay platform. *Inhibition was <50% at the 20 μM dose.

| Kinase Target | IC50 (μM) | Kinase Target | IC50 (μM) | Kinase Target | IC50 (μM) |
|---------------|-----------|---------------|-----------|---------------|-----------|
| hABL1         | >20.0     | hAURKA        | >20.0     | hCLK3         | >20.0     |
| hABL1(F317I)  | >20.0     | hAURKB        | >20.0     | hCLK4         | >20.0     |
| hABL1(F317L)  | >20.0     | hAURKC        | >20.0     | hCSF1R        | >20.0     |
| hABL1(H396P)  | >20.0     | hAXL          | >20.0     | hCSF1R-autoinhib | 7.5* |
| hABL1(pE255K) | >20.0     | bacPKNB       | >20.0     | hCSK          | >20.0     |
| hABL1(pF317I) | >20.0     | hBIKE         | >20.0     | hCSNK1A1      | >20.0     |
| hABL1(pF317L) | >20.0     | hBLK          | >20.0     | hCSNK1A1L     | >20.0     |
| hABL1(pH396P) | >20.0     | hBMPR1A       | >20.0     | hCSNK1D       | >20.0     |
| hABL1(pM351T) | >20.0     | hBMPR1B       | >20.0     | hCSNK1E       | >20.0     |
| hABL1(pQ252H) | >20.0     | hBMPR2        | >20.0     | hCSNK1G1      | >20.0     |
| hABL1(pT315I) | >20.0     | hBMX          | >20.0     | hCSNK1G2      | >20.0     |
| hABL1(pY253F) | >20.0     | hBRAF         | >20.0     | hCSNK1G3      | >20.0     |
| hABL1(Q252H)  | >20.0     | hBRAF(V600E)  | >20.0     | hCSNK2A1      | >20.0     |
| hABL1(T315I)  | >20.0     | hBRSK1        | >20.0     | hCSNK2A2      | >20.0     |
| hABL1-p       | >20.0     | hBRSK2        | >20.0     | hCTK          | >20.0     |
| hABL2         | >20.0     | hBTK          | >20.0     | hDAPK1        | >20.0     |
| hACVR1        | >20.0     | hBUB1         | >20.0     | hDAPK2        | >20.0     |
| hACVR1B       | >20.0     | hCAMK1        | >20.0     | hDAPK3        | >20.0     |
| hACVR2A       | >20.0     | hCAMK1D       | >20.0     | hDCAMKL1      | >20.0     |
| hACVR2B       | >20.0     | hCAMK1G       | >20.0     | hDCAMKL2      | >20.0     |
| hACVR1L       | >20.0     | hCAMK2A       | >20.0     | hDCAMKL3      | >20.0     |
| hADCK3        | >20.0     | hCAMK2B       | >20.0     | hDDR1         | >20.0     |
| hADCK4        | >20.0     | hCAMK2D       | >20.0     | hDDR2         | >20.0     |
| hAKT1         | >20.0     | hCAMK2G       | >20.0     | hDLK          | >20.0     |
| hAKT2         | >20.0     | hCAMK4        | >20.0     | hDMPK         | >20.0     |
| hAKT3         | >20.0     | hCAMKK1       | >20.0     | hDMPK2        | >20.0     |
| hALK          | >20.0     | hCAMKK2       | >20.0     | hDRAK1        | >20.0     |
| hALK(C1156Y)  | >20.0     | hCDKL2        | >20.0     | hDRAK2        | >20.0     |
| hALK(L1196M)  | >20.0     | hCDKL3        | >20.0     | hDYRK1A       | >20.0     |
| hAMPK-alpha1  | >20.0     | hCDKL5        | >20.0     | hDYRK1B       | 14.5*     |
| hAMPK-alpha2  | >20.0     | hCHEK1        | >20.0     | hDYRK2        | >20.0     |
| hANKK1        | >20.0     | hCHEK2        | >20.0     | hEGLFR        | >20.0     |
| hARK5         | >20.0     | hCIT          | >20.0     | hEGLFR(E746-A750del) | >20.0 |
| hASK1         | >20.0     | hCLK1         | >20.0     | hEGLFR(G719C) | >20.0     |
| hASK2         | >20.0     | hCLK2         | >20.0     | hEGLFR(G719S) | >20.0     |
SUPPLEMENTAL TABLE 2 (continued)

| Kinase Target                  | IC50 (μM) | Kinase Target                  | IC50 (μM) |
|-------------------------------|-----------|-------------------------------|-----------|
| hEGFR(L747-E749del, A750P)   | >20.0     | hFGFR1                        | >20.0     |
| hEGFR(L747-S752del, P753S)   | >20.0     | hFGFR2                        | >20.0     |
| hEGFR(L747-T751del,Sins)     | >20.0     | hFGFR3                        | >20.0     |
| hEGFR(L858R)                  | >20.0     | hFGFR3(G697C)                 | >20.0     |
| hEGFR(L858R,T790M)           | >20.0     | hFGFR4                        | >20.0     |
| hEGFR(L861Q)                 | >20.0     | hFGFR4                        | >20.0     |
| hEGFR(S752-I759del)          | >20.0     | hFLT1                         | >20.0     |
| hEGFR(T790M)                 | >20.0     | hFLT1                         | >20.0     |
| hEIF2AK1                     | >20.0     | hFLT3(D835H)                  | >20.0     |
| hEPHA1                       | >20.0     | hFLT3(D835Y)                  | >20.0     |
| hEPHA2                       | >20.0     | hFLT3(ITD)                    | >20.0     |
| hEPHA3                       | >20.0     | hFLT3(K663Q)                  | >20.0     |
| hEPHA4                       | >20.0     | hFLT3(N841I)                  | >20.0     |
| hEPHA5                       | >20.0     | hFLT3(R834Q)                  | >20.0     |
| hEPHA6                       | >20.0     | hFLT3-autoinhibited           | >20.0     |
| hEPHA7                       | >20.0     | hFLT4                         | >20.0     |
| hEPHA8                       | >20.0     | hFRK                          | >20.0     |
| hEPHB1                       | >20.0     | hFYN                          | >20.0     |
| hEPHB2                       | >20.0     | hGAK                          | >20.0     |
| hEPHB3                       | >20.0     | hGCN2(Kin.Dom.2,S808G)        | >20.0     |
| hEPHB4                       | >20.0     | hGRK1                         | >20.0     |
| hEPHB6                       | >20.0     | hGRK4                         | >20.0     |
| hERBB2                       | >20.0     | hGRK7                         | >20.0     |
| hERBB3                       | >20.0     | hGSK3A                        | >20.0     |
| hERBB4                       | >20.0     | hGSK3B                        | >20.0     |
| hERK1                        | >20.0     | hHASPIN                       | >20.0     |
| hERK2                        | >20.0     | hHCK                          | >20.0     |
| hERK3                        | >20.0     | hHIPK1                        | >20.0     |
| hERK4                        | >20.0     | hHIPK2                        | >20.0     |
| hERK5                        | >20.0     | hHIPK3                        | >20.0     |
| hERK8                        | >20.0     | hHIPK4                        | >20.0     |
| hERN1                        | >20.0     | hHPK1                         | >20.0     |
| hFAK                         | >20.0     | hHUNK                         | >20.0     |
| hFER                         | >20.0     | hICK                          | >20.0     |
| hFES                         | >20.0     | hIGF1R                        | >20.0     |
| Kinase Target                        | IC50 (μM) | Kinase Target                        | IC50 (μM) |
|-------------------------------------|-----------|-------------------------------------|-----------|
| hIKK-alpha                          | >20.0     | hLYN                                | >20.0     |
| hIKK-beta                           | >20.0     | hLZK                                | >20.0     |
| hIKK-epsilon                        | >20.0     | hMAK                                | >20.0     |
| hINSR                               | >20.0     | hMAP3K1                             | >20.0     |
| hINSRR                              | >20.0     | hMAP3K15                            | >20.0     |
| hIRAK1                              | >20.0     | hMAP3K2                             | >20.0     |
| hIRAK3                              | >20.0     | hMAP3K3                             | >20.0     |
| hIRAK4                              | >20.0     | hMAP3K4                             | >20.0     |
| hITK                                | >20.0     | hMAP4K2                             | >20.0     |
| hJAK1(JH1 Dom.-catalytic)           | >20.0     | hMAP4K3                             | >20.0     |
| hJAK1(JH2 Dom.-pseudokinase)        | >20.0     | hMAP4K4                             | >20.0     |
| hJAK2(JH1 Dom.-catalytic)           | >20.0     | hMAP4K5                             | >20.0     |
| hJAK3(JH1 Dom.-catalytic)           | >20.0     | hMAPKAPK2                           | >20.0     |
| hJNK1                               | >20.0     | hMAPKAPK5                           | >20.0     |
| hJNK2                               | >20.0     | hMARK1                              | >20.0     |
| hJNK3                               | >20.0     | hMARK2                              | >20.0     |
| hKIT                                | >20.0     | hMARK3                              | >20.0     |
| hKIT(A829P)                         | >20.0     | hMARK4                              | >20.0     |
| hKIT(D816H)                         | >20.0     | hMAST1                              | >20.0     |
| hKIT(D816V)                         | >20.0     | hMEK1                               | >20.0     |
| hKIT(L576P)                         | >20.0     | hMEK2                               | >20.0     |
| hKIT(V559D)                         | >20.0     | hMEK3                               | >20.0     |
| hKIT(V559D,T670I)                   | >20.0     | hMEK4                               | >20.0     |
| hKIT(V559D,V654A)                   | >20.0     | hMEK5                               | >20.0     |
| hKIT-autoinhibited                  | >20.0     | hMEK6                               | >20.0     |
| hLATS1                              | >20.0     | hMELK                                | >20.0     |
| hLATS2                              | >20.0     | hMERTK                              | >20.0     |
| hLCK                                | >20.0     | hMET                                 | >20.0     |
| hLIMK1                              | >20.0     | hMET(M1250T)                         | >20.0     |
| hLIMK2                              | >20.0     | hMET(Y1235D)                         | >20.0     |
| hLKB1                               | >20.0     | hMINK                                | >20.0     |
| hLOK                                | >20.0     | hMKK7                                | >20.0     |
| hLRRK2                              | >20.0     | hMKNK1                              | >20.0     |
| hLRRK2(G2019S)                      | >20.0     | hMKNK2                              | >20.0     |
| hLTK                                | >20.0     | hMLCK                                | >20.0     |
**SUPPLEMENTAL TABLE 2 (continued)**

| Kinase Target | IC50 (μM) | Kinase Target | IC50 (μM) | Kinase Target | IC50 (μM) |
|---------------|-----------|---------------|-----------|---------------|-----------|
| hMLK1         | >20.0     | hp38-delta    | >20.0     | hPIK3CA(Q546K) | >20.0     |
| hMLK2         | >20.0     | hp38-gamma    | >20.0     | hPIK3CB       | >20.0     |
| hMLK3         | >20.0     | hPAK1         | >20.0     | hPIK3CD       | >20.0     |
| hMRCKA        | >20.0     | hPAK2         | >20.0     | hPIK3CG       | >20.0     |
| hMRCKB        | >20.0     | hPAK3         | >20.0     | hPIK4CB       | >20.0     |
| hMST1         | >20.0     | hPAK4         | >20.0     | hPIM1         | >20.0     |
| hMST1R        | >20.0     | hPAK6         | >20.0     | hPIM2         | >20.0     |
| hMST2         | >20.0     | hPLK4         | >20.0     | hPIM3         | >20.0     |
| hMST3         | >20.0     | prstePFCDPK1  | >20.0     | hPIP5K1A      | 16.4      |
| hMST4         | >20.0     | prstePFPPK5   | >20.0     | hPIP5K1C      | >20.0     |
| hMTOR         | >20.0     | hPRKCD        | >20.0     | hPIP5K2B      | >20.0     |
| hMUSK         | >20.0     | hPRKCE        | >20.0     | hPIP5K2C      | >20.0     |
| hMYLK         | >20.0     | hPRKCH        | >20.0     | hPKAC-alpha   | >20.0     |
| hMYLK2        | >20.0     | hPAK7         | >20.0     | hPKAC-beta    | >20.0     |
| hMYLK4        | >20.0     | hPCTK1        | >20.0     | hPKMYT1      | >20.0     |
| hMYO3A        | >20.0     | hPCTK2        | >20.0     | hPKN1        | >20.0     |
| hMYO3B        | >20.0     | hPCTK3        | >20.0     | hPKN2        | >20.0     |
| hNDR1         | >20.0     | hPDGFRA       | >20.0     | hPLK1        | >20.0     |
| hNDR2         | >20.0     | hPDGFRB       | >20.0     | hPLK2        | >20.0     |
| hNEK1         | >20.0     | hPDPK1        | >20.0     | hPLK3        | >20.0     |
| hNEK10        | >20.0     | hPFTAIRE2     | >20.0     | hPLK4        | >20.0     |
| hNEK11        | >20.0     | hPFTK1        | >20.0     | prstePFCDPK1  | >20.0     |
| hNEK2         | >20.0     | hPHKG1        | >20.0     | prstePFPPK5   | >20.0     |
| hNEK3         | >20.0     | hPHKG2        | >20.0     | hPRKCD       | >20.0     |
| hNEK4         | >20.0     | hPIK3C2B      | >20.0     | hPRKCE       | >20.0     |
| hNEK5         | >20.0     | hPIK3C2G      | >20.0     | hPRKCH       | >20.0     |
| hNEK6         | >20.0     | hPIK3CA       | >20.0     | hPRKC1       | >20.0     |
| hNEK7         | >20.0     | hPIK3CA(C420R)| >20.0     | hPRKCQ       | >20.0     |
| hNEK9         | >20.0     | hPIK3CA(E542K)| >20.0     | hPRKD1       | >20.0     |
| hNIK          | >20.0     | hPIK3CA(E545A)| >20.0     | hPRKD2       | >20.0     |
| hNIM1         | >20.0     | hPIK3CA(E545K)| >20.0     | hPRKD3       | >20.0     |
| hNLK          | >20.0     | hPIK3CA(H1047L)| >20.0     | hPRKG1       | >20.0     |
| hOSR1         | >20.0     | hPIK3CA(H1047Y)| >20.0     | hPRKG2       | >20.0     |
| hp38-alpha    | >20.0     | hPIK3CA(1800L)| >20.0     | hPRKR        | >20.0     |
| hp38-beta     | >20.0     | hPIK3CA(M1043I)| >20.0     | hPRKX        | >20.0     |
### SUPPLEMENTAL TABLE 2 (continued)

| Kinase Target                        | IC50 (μM) | Kinase Target                        | IC50 (μM) |
|--------------------------------------|-----------|--------------------------------------|-----------|
| hPRP4                                | >20.0     | hSGK3                                | >20.0     |
| hPYK2                                | >20.0     | hSIK                                 | >20.0     |
| hQSK                                 | >20.0     | hSIK2                                | >20.0     |
| hRAF1                                | >20.0     | hSLK                                 | >20.0     |
| hRET                                 | >20.0     | hSNARK                               | >20.0     |
| hRET(M918T)                          | >20.0     | hSNRK                                | >20.0     |
| hRET(V804L)                          | >20.0     | hSRC                                 | >20.0     |
| hRET(V804M)                          | >20.0     | hSRMS                                | >20.0     |
| hRIOK1                               | >20.0     | hSRPK1                               | >20.0     |
| hRIOK2                               | >20.0     | hSRPK2                               | >20.0     |
| hRIOK3                               | >20.0     | hSRPK3                               | >20.0     |
| hRIPK1                               | >20.0     | hSTK16                               | >20.0     |
| hRIPK2                               | >20.0     | hSTK33                               | >20.0     |
| hRIPK4                               | >20.0     | hSTK35                               | >20.0     |
| hRIPK5                               | >20.0     | hSTK36                               | >20.0     |
| hROCK1                               | >20.0     | hSTK39                               | >20.0     |
| hROCK2                               | >20.0     | hSYK                                 | >20.0     |
| hROS1                                | >20.0     | hTAK1                                | >20.0     |
| hRPS6KA4(Kin.Dom.1-N-term)           | >20.0     | hTAOK1                               | >20.0     |
| hRPS6KA4(Kin.Dom.2-C-term)           | >20.0     | hTAOK2                               | >20.0     |
| hRPS6KA5(Kin.Dom.1-N-term)           | >20.0     | hTAOK3                               | >20.0     |
| hRPS6KA5(Kin.Dom.2-C-term)           | >20.0     | hTBK1                                | >20.0     |
| hRSK1(Kin.Dom.1-N-term)              | >20.0     | hTEC                                 | >20.0     |
| hRSK1(Kin.Dom.2-C-term)              | >20.0     | hTESK1                               | >20.0     |
| hRSK2(Kin.Dom.1-N-term)              | >20.0     | hTGFBR1                              | >20.0     |
| hRSK2(Kin.Dom.2-C-term)              | >20.0     | hTGFBR2                              | >20.0     |
| hRSK3(Kin.Dom.1-N-term)              | >20.0     | hTIE1                                | >20.0     |
| hRSK3(Kin.Dom.2-C-term)              | >20.0     | hTIE2                                | >20.0     |
| hRSK4(Kin.Dom.1-N-term)              | >20.0     | hTLK1                                | >20.0     |
| hRSK4(Kin.Dom.2-C-term)              | >20.0     | hTLK2                                | >20.0     |
| hS6K1                                | >20.0     | hTNIK                                | >20.0     |
| hSBK1                                | >20.0     | hTNK1                                | >20.0     |
| hSGK                                 | >20.0     | hTNK2                                | >20.0     |
| hSgK110                              | >20.0     | hTNNI3K                              | >20.0     |
| hSGK2                                | >20.0     | hTRKA                                | >20.0     |
### SUPPLEMENTAL TABLE 2 (continued)

| Kinase Target                                      | IC50 (μM) |
|---------------------------------------------------|-----------|
| hTRKB                                             | >20.0     |
| hTRKC                                             | >20.0     |
| hTRPM6                                            | >20.0     |
| hTSSK1B                                           | >20.0     |
| hTTK                                              | >20.0     |
| hTXK                                              | >20.0     |
| hTYK2(JH1Dom.-catalytic)                           | >20.0     |
| hTYK2(JH2Dom.-pseudokinase)                        | >20.0     |
| hTYRO3                                            | >20.0     |
| hULK1                                             | >20.0     |
| hULK2                                             | >20.0     |
| hULK3                                             | >20.0     |
| hVEGFR2                                           | >20.0     |
| hVRK2                                             | >20.0     |
| hWEE1                                             | >20.0     |
| hWEE2                                             | >20.0     |
| hWNK1                                             | >20.0     |
| hWNK3                                             | >20.0     |
| hYANK1                                            | >20.0     |
| hYANK2                                            | >20.0     |
| hYANK3                                            | >20.0     |
| hYES                                              | >20.0     |
| hYSK1                                             | >20.0     |
| hYSK4                                             | >20.0     |
| hZAK                                              | >20.0     |
SUPPLEMENTAL TABLE 3: Effect of LLY-507 on the activity of 36 G protein-coupled receptors, using the Eurofins-CEREP pharmacology platform.

| GPCR Target and Assay Mode | % Effect |
|----------------------------|----------|
| h5HT2B Calcium Mobilization Agonist | 3.4% Stimulation @ 13.3 μM |
| h5HT2B Calcium Mobilization Antagonist | 6.8% Inhibition @ 10 μM |
| h5HT2B Calcium Mobilization Potentiation | -7.3% Potentiation @ 10 μM |
| hADORA3 β-arrestin Antagonist | -38.3 Inhibition @ 10 μM |
| hADORA3 cAMP Agonist | 2.6% Stimulation @ 10 μM |
| hADORA3 cAMP Potentiation | -7.3% Potentiation @ 10 μM |
| hADRα1A Calcium Mobilization Agonist | -1.9% Stimulation @ 13.3 μM |
| hADRα1A Calcium Mobilization Antagonist | 98% Inhibition @ 10 μM |
| hADRα1A Calcium Mobilization Potentiation | -7.9% Potentiation @ 10 μM |
| hADRα2A β-arrestin Antagonist | -27.7% Inhibition @ 10 μM |
| hADRα2A cAMP Agonist | 0.2% Stimulation @ 10 μM |
| hADRα2A cAMP Potentiation | -20.5% Potentiation @ 10 μM |
| hADRβ1 β-arrestin Antagonist | -6% Inhibition @ 10 μM |
| hADRβ1 cAMP Agonist | 52.7% Stimulation @ 10 μM |
| hADRβ1 cAMP Potentiation | 32.1% Potentiation @ 10 μM |
| hADRβ2 β-arrestin Antagonist | -15.6% Inhibition @ 10 μM |
| hADRβ2 cAMP Agonist | 0.1% Stimulation @ 10 μM |
| hADRβ2 cAMP Potentiation | -27.2% Potentiation @ 10 μM |
| hD1 β-arrestin Antagonist | 43.4% Inhibition @ 10 μM |
| hD1 cAMP 1% DMSO Potentiation | -19.4% Potentiation @ 10 μM |
| hD1 cAMP Agonist | -6.8% Stimulation @ 10 μM |
| hD2L β-arrestin Antagonist | 29.8% Inhibition @ 10 μM |
| hD2L cAMP Agonist | 10.1% Stimulation @ 10 μM |
| hD2L cAMP Potentiation | -27.5% Potentiation @ 10 μM |
| hH1 β-arrestin Antagonist | 30.1% Inhibition @ 10 μM |
| hH1 Ca Mobilization Agonist | -2.8% Stimulation @ 13.3 μM |
| hH1 Ca Mobilization Potentiation | -8.5% Potentiation @ 10 μM |
| hM2 β-arrestin Agonist | 5.1% Stimulation @ 10 μM |
| hM2 β-arrestin Antagonist | 36.3% Inhibition @ 10 μM |
| hM2 β-arrestin Potentiation | -32.2% Potentiation @ 10 μM |
| hM3 β-arrestin Antagonist | -23.6% Inhibition @ 10 μM |
| hM3 Calcium Mobilization Agonist | -1.4% Stimulation @ 13.3 μM |
| hM3 Calcium Mobilization Potentiation | -18% Potentiation @ 10 μM |
| hOPRm1 β-arrestin Antagonist | -3.9% Inhibition @ 1 μM |
| hOPRm1 cAMP Agonist | 7.4% Stimulation @ 1 μM |
| hOPRm1 cAMP Potentiation | 9.6% Potentiation @ 1 μM |
**SUPPLEMENTAL TABLE 4:** Effect of LLY-507 against 15 nuclear hormone receptors and 3 cytochrome p450 enzymes, using the Eurofins-CEREP pharmacology platform.

| Nuclear Hormone Receptors | Relative IC50 (μM) | Cytochrome p450 Enzymes | % inhibition @ 10 μM |
|---------------------------|--------------------|-------------------------|----------------------|
| hERα                      | >10                | CYP2D6                  | 61.2                 |
| hERβ                      | >10                | CYP2C9                  | 34.2                 |
| hFXR                      | >10                | CYP3A4                  | 69.1                 |
| hLXRα                     | >10                |                         |                      |
| hLXRβ                     | >10                |                         |                      |
| hPPARα                    | >10                |                         |                      |
| hPPARδ                    | >10                |                         |                      |
| hPPARγ                    | >10                |                         |                      |
| hRARα                     | >10                |                         |                      |
| hRARβ                     | >10                |                         |                      |
| hRARγ                     | >10                |                         |                      |
| hRXRα                     | >10                |                         |                      |
| hTRα1                     | >10                |                         |                      |
| hTRβ1                     | >10                |                         |                      |
| hVDR                      | >10                |                         |                      |
SUPPLEMENTAL FIGURE 5: Effect of LLY-507 on cellular post-translational modifications on histone H3 following treatment with LLY-507, as measured by mass spectrometry. Un, unmethylated; me1, mono-methylated; me2, di-methylated; me3, tri-methylated; ac, acetylated.

### A

| Histone H3 Peptide: TKQTAR | Relative abundance | Fold difference |
|----------------------------|--------------------|-----------------|
|                            | Vehicle            | LLY-507, 5 μM   | LLY-507/Vehicle | t-test |
| H3 K4un                    | 85.5 ± 0.9         | 83.8 ± 1.7      | 1.0            | 0.189  |
| H3 K4me1                   | 10.5 ± 0.3         | 13.0 ± 1.0      | 0.8            | 0.077  |
| H3 K4me2                   | 2.6 ± 0.6          | 2.1 ± 0.4       | 1.2            | 0.080  |
| H3 K4me3                   | 1.4 ± 0.3          | 1.0 ± 0.2       | 1.3            | 0.020  |

| Histone H3 Peptide: KSTGGKAPR | Relative abundance | Fold difference |
|--------------------------------|--------------------|-----------------|
|                                | Vehicle            | LLY-507, 5 μM   | LLY-507/Vehicle | t-test |
| H3 K9un, K14un                | 10.2 ± 0.3         | 10.9 ± 1.4      | 0.9            | 0.496  |
| H3 K9me1, K14un               | 7.6 ± 1.2          | 9.7 ± 2.4       | 0.8            | 0.374  |
| H3 K9me2, K14un               | 26.7 ± 0.9         | 24.8 ± 0.2      | 1.1            | 0.043  |
| H3 K9me3, K14un               | 18.6 ± 0.6         | 17.4 ± 0.4      | 1.1            | 0.019  |
| H3 K9ac, K14un                | 2.1 ± 0.9          | 2.5 ± 0.6       | 0.9            | 0.697  |
| H3 K9un, K14ac                | 4.3 ± 0.7          | 4.5 ± 0.5       | 1.0            | 0.700  |
| H3 K9me1, K14ac               | 6.2 ± 0.7          | 8.0 ± 0.7       | 0.8            | 0.148  |
| H3 K9me2, K14ac               | 16.6 ± 0.9         | 15.3 ± 2.8      | 1.1            | 0.528  |
| H3 K9me3, K14ac               | 7.0 ± 0.2          | 6.3 ± 1.3       | 1.1            | 0.453  |
| H3 K9ac, K14ac                | 0.7 ± 0.1          | 0.8 ± 0.1       | 0.9            | 0.057  |

| Histone H3 Peptide: KSAPA1GGVKPHR | Relative abundance | Fold difference |
|-----------------------------------|--------------------|-----------------|
|                                   | Vehicle            | LLY-507, 5 μM   | LLY-507/Vehicle | t-test |
| H3 K27un, K36un                   | 6.1 ± 0.9          | 5.2 ± 0.8       | 1.2            | 0.377  |
| H3 K27un, K36me1                  | 2.1 ± 0.3          | 2.3 ± 0.8       | 0.9            | 0.726  |
| H3 K27me1, K36un                  | 9.0 ± 0.6          | 10.7 ± 1.4      | 0.8            | 0.299  |
| H3 K27me2, K36un                  | 26.0 ± 0.8         | 22.1 ± 1.8      | 1.2            | 0.098  |
| H3 K27un, K36me2                  | 5.6 ± 0.05         | 4.9 ± 0.5       | 1.1            | 0.165  |
| H3 K27me3, K36un                  | 7.5 ± 0.3          | 6.7 ± 0.5       | 1.1            | 0.135  |
| H3 K27ac, K36un                   | 0.1 ± 0.01         | 0.1 ± 0.004     | 1.0            | 0.712  |
| H3 K27me1, K36me1                 | 5.1 ± 0.1          | 6.9 ± 2.3       | 0.7            | 0.297  |
| H3 K27me2, K36me1                 | 16.5 ± 0.8         | 15.2 ± 2.3      | 1.1            | 0.423  |
| H3 K27me1, K36me2                 | 11.6 ± 0.5         | 14.9 ± 1.7      | 0.8            | 0.094  |
| H3 K27me2, K36me2                 | 3.4 ± 0.3          | 3.1 ± 1.1       | 1.1            | 0.639  |
| H3 K27me3, K36me1                 | 3.2 ± 0.1          | 3.3 ± 0.7       | 1.0            | 0.823  |
| H3 K27me1, K36me3                 | 3.2 ± 0.1          | 4.1 ± 0.4       | 0.8            | 0.050  |
| H3 K27me3, K36me2                 | 0.6 ± 0.04         | 0.5 ± 0.2       | 1.2            | 0.436  |

| Histone H3 Peptide: EIAQDFKTDLR | Relative abundance | Fold difference |
|----------------------------------|--------------------|-----------------|
|                                  | Vehicle            | LLY-507, 5 μM   | LLY-507/Vehicle | t-test |
| H3 K79un                         | 78.6 ± 4.1         | 74.8 ± 2.3      | 1.1            | 0.128  |
| H3 K79me1                        | 16.2 ± 3.3         | 17.1 ± 0.8      | 0.9            | 0.640  |
| H3 K79me2                        | 5.1 ± 0.8          | 8.1 ± 2.3       | 0.6            | 0.105  |
**SUPPLEMENTAL FIGURE 5 (continued):** Effect of LLY-507 on cellular post-translational modifications on histone H4 following treatment with LLY-507, as measured by mass spectrometry. Un, unmethylated; me1, mono-methylated; me2, di-methylated; me3, tri-methylated; ac, acetylated.

| Histone H4 peptide: GKKGKGLGKGGAKR | Relative abundance | Fold difference |
|--------------------------------------|-------------------|-----------------|
|                                      | Vehicle           | LLY-507, 5 µM   | LLY-507/vehicle | t-test |
| H4 K5un, K8un, K12un, K16un         | 37.8 ± 5.6        | 36.4 ± 3.4      | 1.04           | 0.778  |
| H4 K5ac, K8un, K12un, K16un         | 2.9 ± 1.9         | 2.6 ± 1.6       | 1.12           | 0.873  |
| H4 K5un, K8ac, K12un, K16un         | 7.0 ± 4.8         | 11.6 ± 2.7      | 0.61           | 0.309  |
| H4 K5un, K8un, K12ac, K16un         | 17.9 ± 7.2        | 16.7 ± 7.4      | 1.07           | 0.859  |
| H4 K5un, K8un, K12un, K16ac         | 19.2 ± 9.4        | 18.0 ± 4.5      | 1.06           | 0.884  |
| H4 K5ac, K8ac, K12un, K16un         | 0.5 ± 0.3         | 0.4 ± 0.1       | 1.26           | 0.625  |
| H4 K5ac, K8un, K12ac, K16un         | 3.0 ± 1.7         | 1.6 ± 0.7       | 1.90           | 0.273  |
| H4 K5ac, K8un, K12un, K16ac         | 0.6 ± 0.5         | 1.6 ± 0.7       | 0.38           | 0.201  |
| H4 K5un, K8ac, K12ac, K16un         | 1.5 ± 0.2         | 1.5 ± 0.2       | 0.98           | 0.854  |
| H4 K5un, K8ac, K12ac, K16ac         | 2.9 ± 1.4         | 2.7 ± 0.6       | 1.06           | 0.772  |
| H4 K5un, K8un, K12ac, K16ac         | 3.7 ± 1.0         | 4.3 ± 0.2       | 0.87           | 0.361  |
| H4 K5ac, K8ac, K12ac, K16un         | 0.4 ± 0.1         | 0.3 ± 0.1       | 1.31           | 0.199  |
| H4 K5ac, K8ac, K12un, K16ac         | 0.5 ± 0.2         | 0.4 ± 0.1       | 1.11           | 0.737  |
| H4 K5ac, K8un, K12ac, K16ac         | 0.4 ± 0.05        | 0.4 ± 0.1       | 1.01           | 0.870  |
| H4 K5un, K8ac, K12ac, K16ac         | 1.4 ± 0.5         | 1.2 ± 0.2       | 1.19           | 0.381  |
| H4 K5un, K8ac, K12ac, K16ac         | 0.2 ± 0.1         | 0.3 ± 0.1       | 0.70           | 0.544  |

**Histone H4 Peptide: KVLR**

| Histone H4 | Relative abundance | Fold difference |
|------------|--------------------|-----------------|
|            | Vehicle            | LLY-507, 5 µM   | LLY-507/vehicle | t-test |
| H4 K20un   | 10.0 ± 0.9         | 10.2 ± 2.3      | 0.98           | 0.907  |
| H4 K20me1  | 27.4 ± 8.0         | 31.0 ± 5.5      | 0.88           | 0.627  |
| H4 K20me2  | 61.8 ± 8.8         | 58.0 ± 7.7      | 1.07           | 0.669  |
| H4 K20me3  | 0.8 ± 0.1          | 0.8 ± 0.1       | 0.97           | 0.796  |
SUPPLEMENTAL METHODS

Chemical synthesis of LLY-507

Route to LLY-507:

1. KOH, DMSO, r.t., 2h to overnight

2. MeCl, DCM, TEA, 0°C, 2h

3. BINAP, Pd2(dba)3, NaOtBu, toluene, 100°C, 3h

4. Pd(dppe)Cl2, K2CO3, DMF, 100°C, overnight

5. TFA/DCM(1:5), r.t., 2h

6. DIPEA, CH3CN, 100°C overnight

7. NaOH, THF/H2O, r.t., 2h
Step 1-Preparation of compound 3

A mixture of 3-methyl-1H-indole (70.00 g, 533.64 mmol) and KOH (205.63 mL, 299.40 g, 5.34 mol) in DMSO (700 mL) was stirred at 16 °C for 2 hr. Then to the mixture was added 2-bromoethanol (37.83 mL, 66.69 g, 533.64 mmol) in one portion.

The mixture was stirred at 16 °C for 24 hr when TLC showed the reaction was complete.

The reaction mixture was poured into 3.0L of ice/water and extracted with EtOAc (500 mL x 3). The combined organic phase was concentrated and purified by column (petroleum ether/ EtOAc = 5/1 to 3/1) to give 2-(3-methylindol-1-yl)ethanol (46.50 g, 265.37 mmol; 49.73% yield) as brown slurry.
Step 2-Preparation of compound 4

At 0 °C, to a mixture of 2-(3-methylindol-1-yl)ethanol (46.00 g, 262.52 mmol) and triethylamine (73.08 mL, 53.13 g, 525.03 mmol) in dichloromethane (600 mL) was added methanesulfonyl chloride (24.38 mL, 36.09 g, 315.02 mmol) dropwise.

The mixture was stirred at room temperature for 2 hrs.

LCMS showed the reaction was complete. The mixture was poured into 100 mL of ice water, extracted with dichloromethane (100 mL x 2).

The combined organic phase was dried over Na$_2$SO$_4$, and concentrated to give the crude product 2-(3-methylindol-1-yl)ethyl methanesulfonate (55.00 g, 217.12 mmol; 82.70% yield), which was used for next step directly.

Step 3-Preparation of compound 7

A mixture of 1-bromo-2-iodo-benzene (90.91 mL, 200.00 g, 706.95 mmol) and tert-butyl piperazine-1-carboxylate (158.00 g, 848.34 mmol) and sodium 2-methylpropan-2-olate (101.91 g, 1.06 mol) and (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one; palladium (32.37 g, 35.35 mmol) and (±)-2,2'-
Bis(diphenylphosphino)-1,1'-binaphthalene (44.02 g, 70.70 mmol) in toluene (1500 mL) was stirred at 100 °C under N₂ for 4 hrs. TLC showed the reaction was complete.

The mixture was diluted with EtOAc and water then filtered. The filtrate was extracted with EtOAc and concentrated. The residue was purified with column to give tert-butyl 4-(2-bromophenyl)piperazine-1-carboxylate as a yellowish slurry.

Another lot from 150 g of 1-bromo-2-iodo-benzene has been run separately, which was worked up together with the first batch. Total yield 120.00 g, 351.66 mmol; was obtained with 28.4% yield.

**Step 4-Preparation of compound 9**

![Chemical structure](image)

A mixture of methyl 3-cyanobenzoate (100.00 g, 620.51 mmol) 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (87.35 g, 682.56 mmol) (1Z,5Z)-cycloocta-1,5-diene;2,4-dimethyl-BAHalbicyclo[1.1.0]butane (8.23 g, 12.41 mmol) 4-tert-butyl-2-(4-tert-butyl-2-pyridyl)pyridine (5.00 g, 18.62 mmol) in hexane (750 mL) was stirred at 70 °C under N₂ protection for 3 hrs. LCMS showed the reaction was complete.

The mixture was poured to a silica bed. Eluted with petroleum: ethyl acetate (5:1) and concentrated to give the product methyl 3-cyano-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (100.00 g, 348.29 mmol; 56.13% yield) as a white solid.

**Step 5-Preparation of compound 10**

![Chemical structure](image)

Methyl 3-cyano-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (100.00 g, 348.29 mmol), tert-butyl 4-(2-bromophenyl)piperazine-1-carboxylate (118.85 g, 348.29 mmol), Pd(dppf)Cl₂ (25.84 g, 34.83 mmol), K₂CO₃ (39.62 mL, 96.27 g, 696.58 mmol) in DMF (1000 mL) were de-gassed and then heated to
80 °C for 4 hrs. TLC (petroleum ether:EtOAc=8:1) showed the starting material was consumed completely. The reaction mixture was poured into H$_2$O (300 mL). The mixture was extracted with EtOAc (3 x 250 mL). The organic phase was washed with saturated brine (300 mL), dried over anhydrous MgSO$_4$, concentrated in vacuum to give a residue, which was pre-purified by column chromatography to afford the pure tert-butyl-4-[2-(3-cyano-5-methoxycarbonyl-phenyl) phenyl]piperazine-1-carboxylate (80.00 g, 189.80 mmol; 54.50% yield) as a slurry.

**Step 6-Preparation of compound 11**

![Diagram of compounds 10 and 11]

To a mixture of compound 10 (80.00 g, 189.80 mmol) in dichloromethane (1 L) was added TFA (151.49 g, 1.33 mol) in portionwise at r.t. under N$_2$. The mixture was stirred at r.t. for 16 hrs. TLC showed the reaction was completed. The mixture was poured into ice-water (1000 mL) and adjust with NaHCO$_3$ until pH = 9. The aqueous phase was extracted with dichloromethane (400 mL x 3). The combined organic phase was washed with saturated brine (200 mL x 2), dried with anhydrous Na$_2$SO$_4$, filtered and concentrated in vacuum to afford methyl 3-cyano-5-(2-piperazin-1-ylphenyl)benzoate (80.00 g, 211.59 mmol; 111.48% yield) as yellow solid, the crude was used for next step directly.

**Step 7-Preparation of compound 12**

![Diagram of compounds 11 and 12]

To a mixture of 2-(3-methylindol-1-yl)ethyl methanesulfonate (47.29 g, 186.70 mmol) and methyl 3-cyano-5-(2-piperazin-1-ylphenyl)benzoate (60.00 g, 186.70 mmol) in acetonitrile (200 mL) was added diisopropylethylamine (65.04 mL, 48.26 g, 373.40 mmol) in one portion at r.t.. The mixture was stirred
at 100 °C for 16 hrs. TLC showed the reaction was complete and was then concentrated in vacuum. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, petroleum ether/ethyl acetate/ dichloromethane = 4/1/1) to afford methyl 3-cyano-5-[2-[4-[2-(3-methylindol-1-yl)ethyl]-piperazin-1-yl]phenyl]benzoate (15.00 g, 31.34 mmol; 16.79% yield) as yellow solid, and 30 g crude product with the purity is ~50%.

**Step 8-Preparation of compound 13**

To a mixture of methyl 3-cyano-5-[2-[4-[2-(3-methylindol-1-yl)ethyl]-piperazin-1-yl]phenyl]benzoate (15.00 g, 31.34 mmol) in THF (100 mL) was added NaOH (3.76 g, 94.02 mmol) in 50mL of water in one portion at r.t. The mixture was heated to 40 °C and stirred for 2 hrs. TLC showed the reaction was completed. The mixture was cooled to r.t. and concentrated in reduced pressure at 45 °C. The residue was poured into ice-water (w/w = 1/1) (1500 mL) and adjusted with 2M of HCl to pH=5. The solid formed was dried in vacuo to afford 3-cyano-5-[2-[4-[2-(3-methylindol-1-yl)ethyl]-piperazin-1-yl]phenyl]benzoic acid (14.00 g, 30.14 mmol; 96.16% yield) as a yellowish solid, which was used for next step without purification.

**Step 9-Preparation of LLY-507**

To a mixture of 5-cyano-2'-{(4-(2-(3-methyl-1H-indol-1-yl)ethyl)piperazin-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (15.00 g, 32.29 mmol) and 3-pyrrolidin-1-ylpropan-1-amine (4.14 g, 32.29 mmol) in dichloromethane (20 mL), was added EDCI (7.43 g, 38.75 mmol) and HOBT (5.24 g, 38.75 mmol) and DIPEA (11.25 mL, 8.35 g, 64.58 mmol) in one portion at r.t. The mixture was stirred at r.t. for 16 hrs. HPLC showed the reaction was completed. The mixture was poured into ice-water (150 mL) and extracted with dichloromethane (30 mL x 3). The combined organic phase was washed with saturated brine (50 mL), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was
purified by silica gel chromatography (column height: 250 mm, 100-200 mesh silica gel, dichloromethane/MeOH = 30/1, 10/1) to afford 3-cyano-5-[2-[4-[2-(3-methylindol-1-yl)ethyl]piperazin-1-yl]phenyl]-N-(3-pyrrolidin-1-ylpropyl)benzamide (5.20 g, 9.05 mmol; 28.02% yield) as yellow solid, and some crude product3.0 g with the purity is ~50%.

Note: Due to the high polarity of the product, using DMF as solvent, the yield should be improved significantly.