

## Supplementary Information

### Sigma-2 Receptor Ligand Binding Modulates Association between TSPO and TMEM97

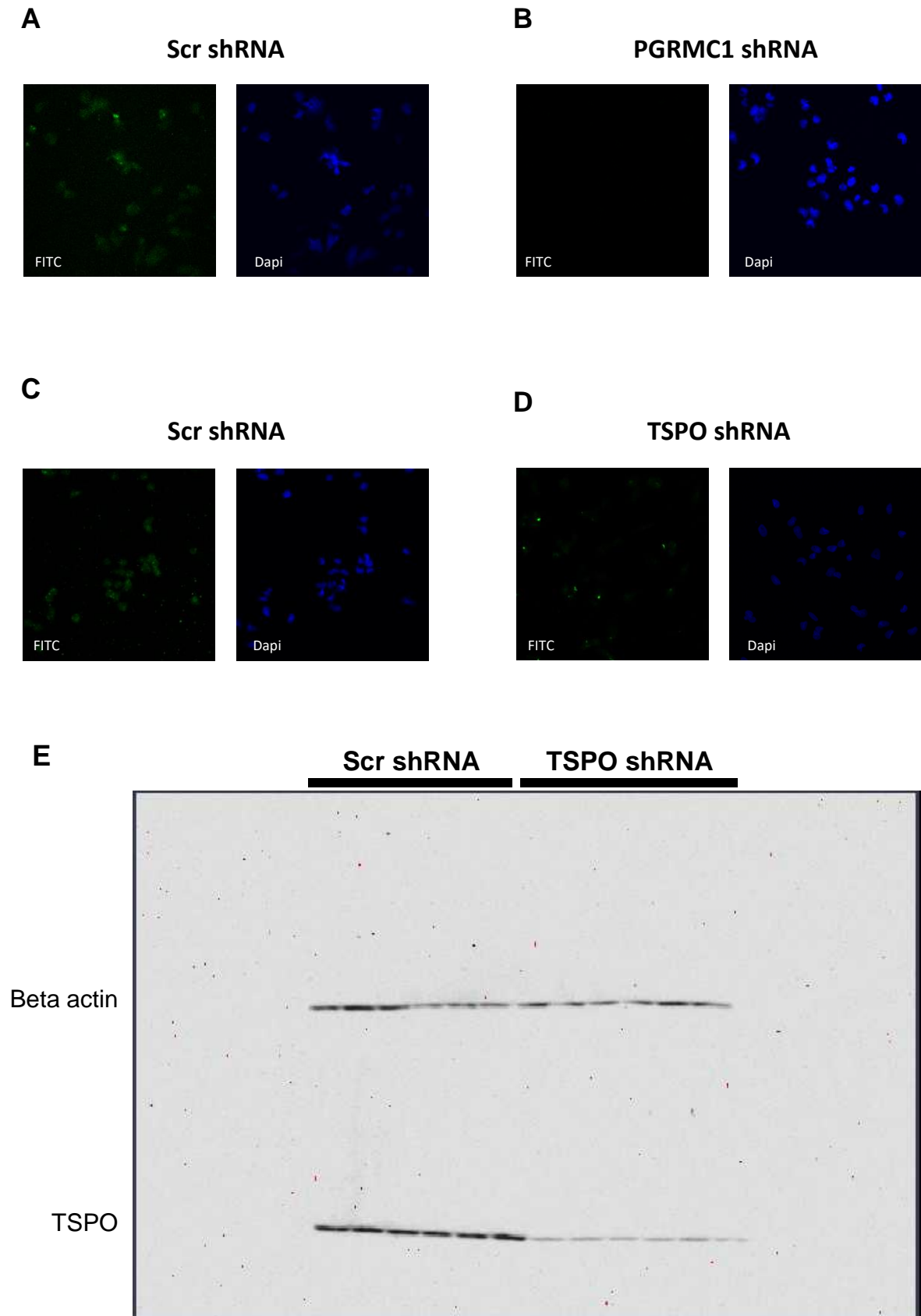
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**Figure S1. PGRMC1 and TSPO knockout by shRNA.**

(A) Represents immunohistochemistry (IHC) analysis of PGRMC1 expression in Scr shRNA MP cell lines. Images were captured by confocal microscopy using primary PGRMC1 antibody at 1:100 concentration and

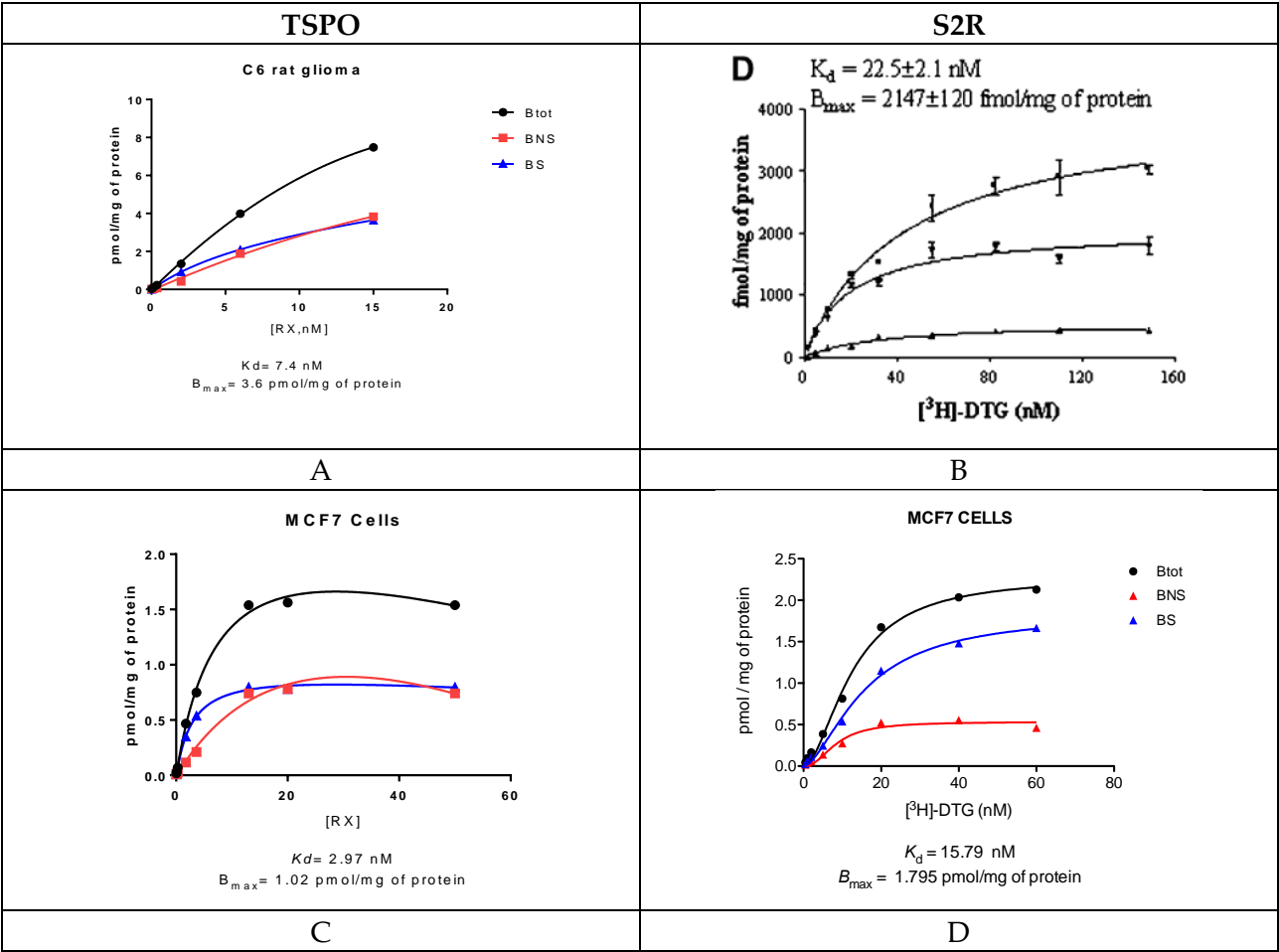
FITC-conjugated secondary antibody (Sigma, F8521). Blue represents DAPI staining of DNA. Each discrete blue patch represents one cell nucleus, imaged at 20x magnification.

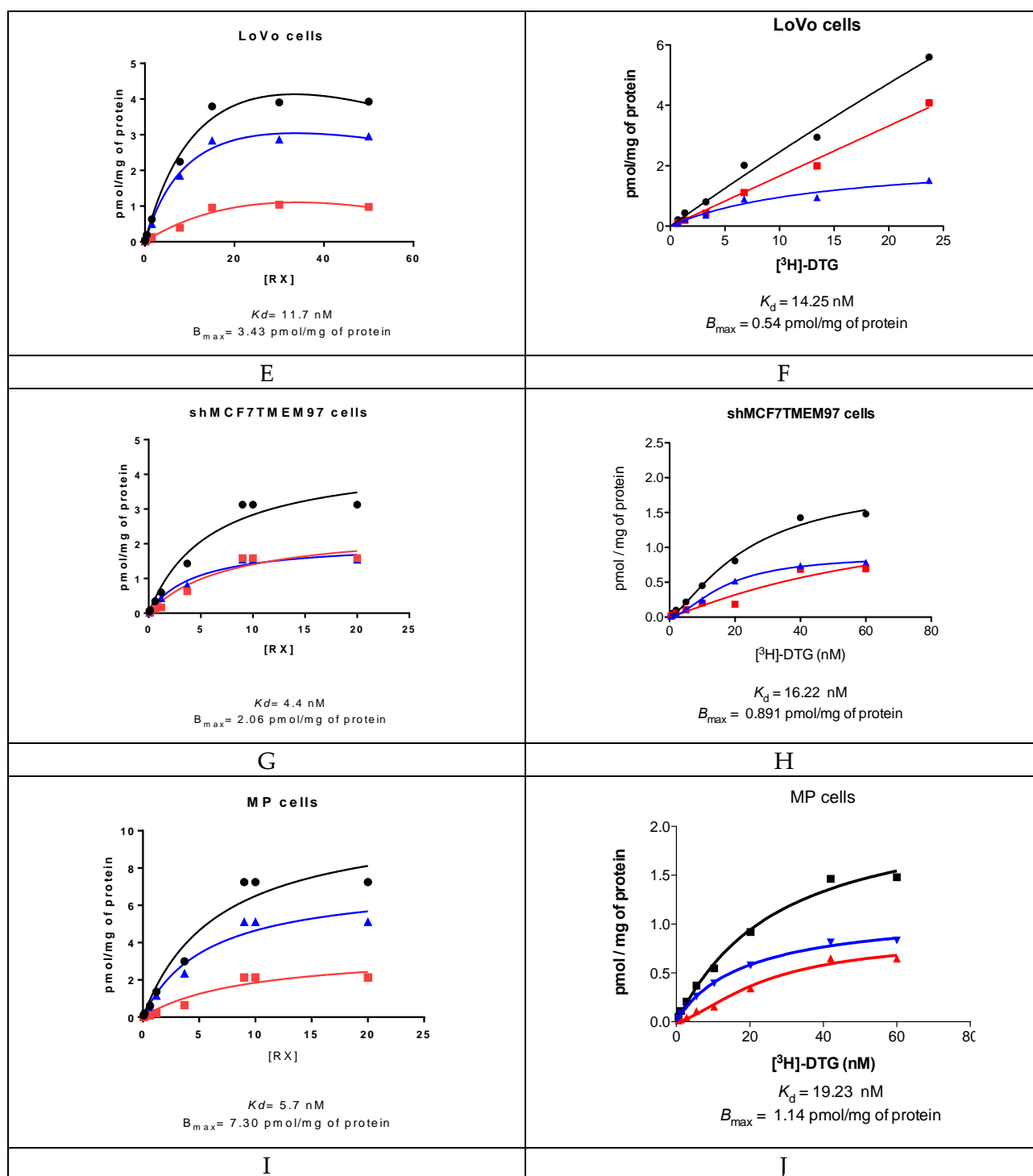
(B) Represents IHC analysis after attenuation the level of PGRMC1 by shRNA using similar antibodies, incubation conditions and confocal microscopy setting. Scale follows (A).

(C) Illustrates the level of TSPO in MP cell lines using goat anti-TSPO primary antibody (Invitro Technologies, NOVNB10041398) at 1:100 and FITC anti-goat secondary antibody. Scale follows (A).

(D) A negative control that shows the attenuation of TSPO by shRNA. Again, similar conditions were used for signal detection.

(E) Image of the original Western blot that was quantified to generate Figure 1C.



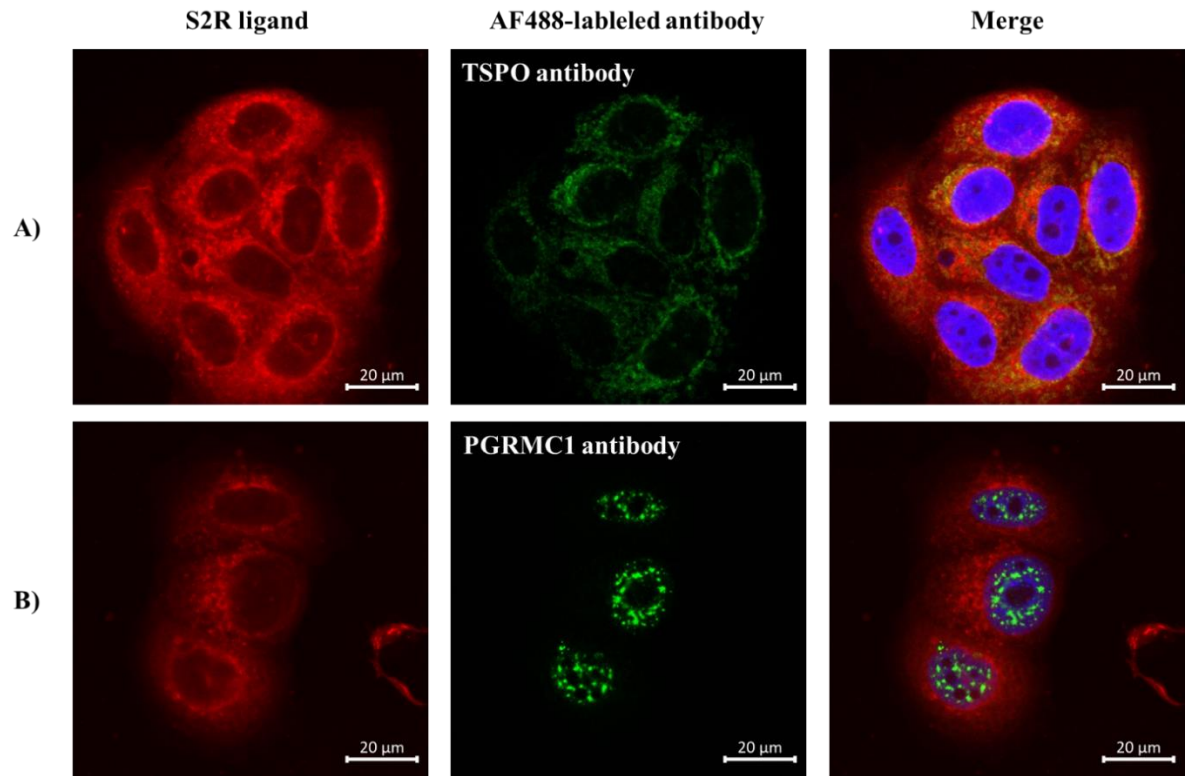


**Figure S2.** Saturation analysis with the appropriate radioligand of TSPO and S2R in membrane preparations from rat C6 (A and B), MCF7 (C and D), LoVo (E and F), shMCF7TMEM97 (G and H) and MP (Mia PaCa-2) (I and J) cell lines.

(A, C, E, G and I) For TSPO saturation binding assays  $[^3\text{H}]\text{PK1195}$  has been used as radioligand and  $10 \mu\text{M}$  PK1195 to measure the no-specific binding;

(B, D, F, H and J) For S2R saturation binding assays  $[^3\text{H}]\text{DTG}$  has been used as radioligand and  $10 \mu\text{M}$  DTG to measure the no-specific binding.

The dissociation constant  $K_d$  and the receptor density (ligand binding sites)  $B_{\max}$  were measured by the nonlinear fitting of the specific binding vs RX concentration using Prism software.



**Figure S3.** Representative confocal microscopy images show the binding site of the CY5-labeled S2R ligand, co-stained with (A) TSPO- as well as (B) PGRMC1-specific antibodies in MCF7 cells. Cells were pre-incubated with 10 μM of the selective S1R agonist (+)-pentazocine for 2 h to mask residual S1R expression. This was followed by 1 h incubation with 5 μM for the S2R ligand (shown in red). Then cells were fixed with paraformaldehyde, stained for TSPO or PGRMC1 expression (shown in green) as well as nuclei (by DAPI, shown in blue) and analyzed by confocal microscopy (scale bar: 20 μm).

**Table S1. STR profiling of MCF7 and MP cell lines.** STR typing of DNA obtained from 2 samples collected was performed with the NGM amplification kit (TFS; see Materials and methods). The effectiveness of autosomal STR typing, expressed as the number of successfully typed loci, is shown in the table above. The STRs characterized in ATCC are bold.

Locus designation NGM Select kit	Chromosome location	Allelels MIACAPA-2	Allelels MCF7
D10S1248	10q26.3	14/15	14/14
<b>vWA</b>	<b>12p13.31</b>	<b>15/15</b>	<b>14/15</b>
<b>D16S539</b>	<b>16q24.1</b>	<b>10/13</b>	<b>11/12</b>
<b>D2S1338</b>	<b>2q35</b>	<b>25/25</b>	<b>21/23</b>
<b>Amelogenin</b>	<b>X:p22.1-22.3</b> <b>Y:p11.2</b>	<b>XX</b>	<b>XX</b>
D8S1179	8q24.13	16/19	10/14
D21S11	21q11.2-q21	29/31.2	30/30
D18S51	18q21.33	12/12	14/14
D22S1045	22q12.3	16/16	15/16
D19S433	19q12	15/15	13/14
<b>TH01</b>	<b>11p15.5</b>	<b>9/10</b>	<b>6/6</b>
FGA	4q28	22/22	23/25
D2S441	2p14	14/15	10/14
D3S1358	3p21.31	16/16	16/16
D1S1656	1q42.2	15/17.3	11/15.3
D12S391	12p13.2	19/19	18/20
SE33	6	16/16	16/18