

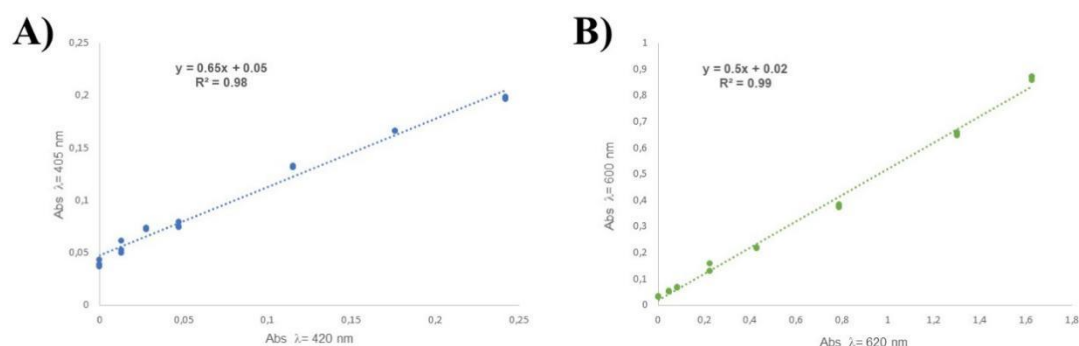
Supplementary files

Drug screening targeting TREM2-TYROBP transmembrane binding

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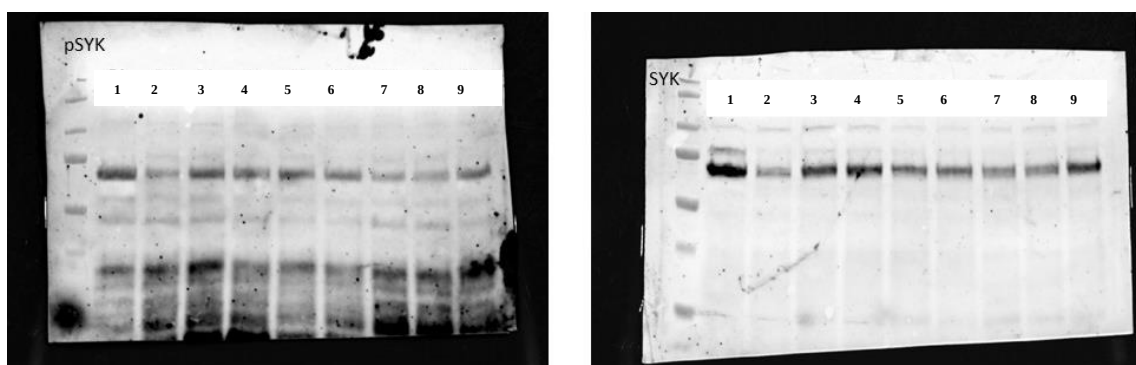
Supplementary results

Supplementary Figure 1. Beta-galactosidase assay absorbance's correlation.



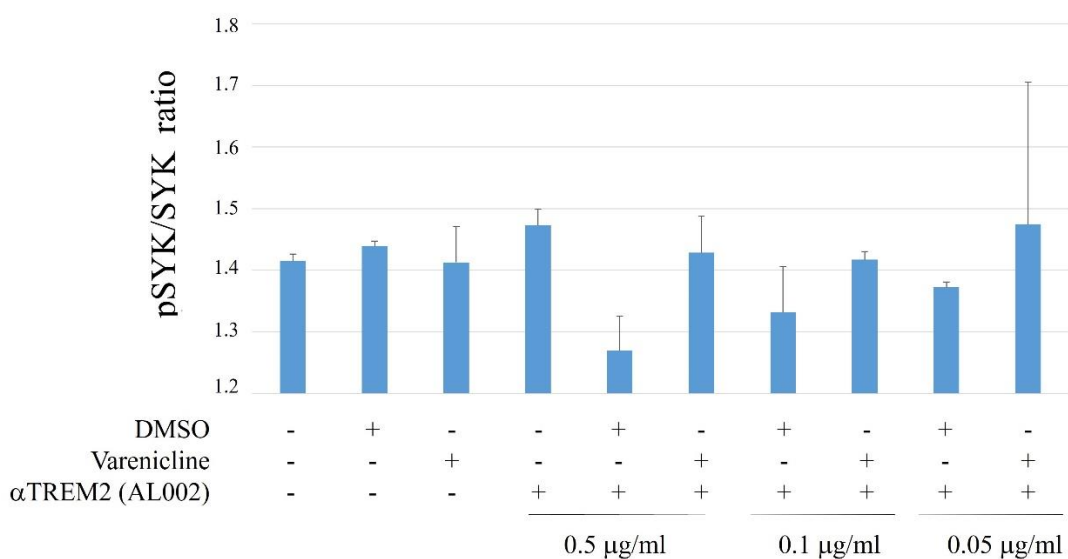
Supplementary Figure 1. Beta-galactosidase assay absorbance's correlation. A) Correlation between absorbance at $\lambda = 420$ nm (X axis) and $\lambda = 405$ nm (Y axis) taken by the ELISA plate reader. Equation and correlation coefficient (R^2) can be found in the top left corner. B) Correlation between absorbances at $\lambda = 600$ nm (X axis) and $\lambda = 620$ nm (Y axis) taken by the ELISA plate reader. Equation and correlation coefficient (R^2) can be found in the top left corner. Statistical analysis showed significant results ($p < 0.001$) when performing Spearman's test.

Supplementary Figure 2. Western blot membranes against SYK and pSYK.



Supplementary Figure 2. Western blot membranes against SYK (A) and pSYK. Proteins extracted from; in lane 1: unstimulated cells with 1% DMSO, lines 2-3: α TREM2 stimulated cells with DMSO, lines 4-6: α TREM2 stimulated cells with varenicline 150 μ M and lines 7-9: α TREM2 stimulated cells with parbimostat 150 μ M

Supplementary Figure 3. pSYK/SYK ratio measured with cell-based ELISA



Supplementary Figure 3. pSYK/SYK ratio measured with cell-based ELISA. HCM3 cells were stimulated with different concentrations of AL002 with 1% DMSO or 150 µM Varenicline. Barrs represent averages (n=3) with errors lines the standard deviation.

Antagonists candidates				Agonists candidates			
Drug	Average	StdDev	B2H confirmation	Drug	Average	StdDev	B2H confirmation
P163-D9	0.2	0.03	Yes	P161-E6	1.16	0.03	-
P163-F6	0.5	0.05	Yes	P161-H6	1.17	0.07	-
P162-G11	0.5	0.10	-	P164-G6	1.17	0.04	-
P161-A6	0.68	0.16	-	P162-E3	1.18	0.03	-
P161-G10	0.74	0.05	Yes	P162-G6	1.20	0.03	-
P162-H8	0.8	0.18	-	P162-D4	1.25	0.07	-
P162-D5	0.76	0.05	-	P163-C6	1.3	0.25	Yes
P162-B8	0.8	0.10	-	P162-G4	1.30	0.08	-
P162-H8	0.8	0.08	-	P164-H6	1.31	0.04	-
P162-G7	0.8	0.03	-	P161-A5	1.33	0.14	Yes
P162-B8	0.8	0.03	-	P164-H5	1.36	0.15	-
P164-E9	0.90	0.14	-	P161-E8	1.36	0.09	-
P162-D4	0.9	0.18	-	P164-G8	1.45	0.14	-
			-	P161-F7	1.46	0.06	-
			-	P164-H8	1.49	0.08	-
			-	P162-F7	1.5	0.31	Yes
			-	P164-H4	1.50	0.14	-
			-	P164-F10	1.58	0.04	Yes
			-	P164-F6	1.59	0.04	-
			-	P164-E5	1.64	0.03	Yes
			-	P164-C6	1.66	0.15	Yes
			-	P161-H4	1.75	0.25	Yes
			-	P164-C11	2.17	0.37	Yes
			-	P161-G9	2.86	0.26	Yes
			-	P161-H10	3.30	1.02	Yes

Supplementary table 1. Initially selected candidate summary. Induction fold -average and standard deviation- of the different candidate drugs. Basal levels were obtained from bacterial cultures in LB with 1% DMSO (average 1.0 ± 0.14). As positive control reference, bacterial cultures grown with DMSO plus IPTG was used (average 1.46 ± 0.72). Only those independently confirmed in an extra beta-galactosidase assay (B2H confirmation column) underwent the specificity assay.

Supplementary methods

5 The complete protein used as bait is translated from pKNT25 of the in-frame cloned BATCH pKTN25 plasmid, at the BamHI and HindIII sites, generating the full construct Pf3: TYROBPTMD:

MTMITPSLQSVITDVTGQLTAVQADITTIGGGVLAGIVMGDLVLTVLIALAVYFLGGGDPRVPSS
NSMTMQQSHQAGYANAADRESGIPAAVLDGIKAVAKEKNATLMFRLVNPSTSLIAEGVATKG
LGVHAKSSDWGLQAGYIPVNPNSKLFGRAPVIAADNDVNSSLAHGHTAVDLTSLKERLDYL
10 RQAGLVTGMADGVVASNHAGYEQFEFRVKETSDGRYAVQYRRKGGDDFEAVKVIGNAAGIPL
TADIDMFAIMPHLSNFRDSARSSVTSGDSVTDYLRTRRAAPSI

The full protein translated as prey from the plasmid pUT18 upon an equivalent cloning generated the Pf3: TREM2TMD construct:

MTMITPSLQSVITDVTGQLTAVQADITTIGGSILLLLACIFLIKILAASALWAGGGDPRVPSSNSAA
SEATGGLDRERIDLLWKIARAGARSAVGTEARRQFRYDGMNIGVITDFELEVNRNALNRRRAHAV
15 GAQDVVQHGTEQNNPFPEADEKIFVVSATGESQMLTRGQLKEYIGQQRGEGYVIFYENRAYGVA
GKSLFDDGLGAAPGVPSGRSKFSPDVLETVPASPGLRRPSLGAVERQSI