Online Data Supplement

Endogenous IL-11 Signaling Is Essential in Th2- and IL-13-Induced Inflammation and Mucus Production

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Online Supplemental Figure E1

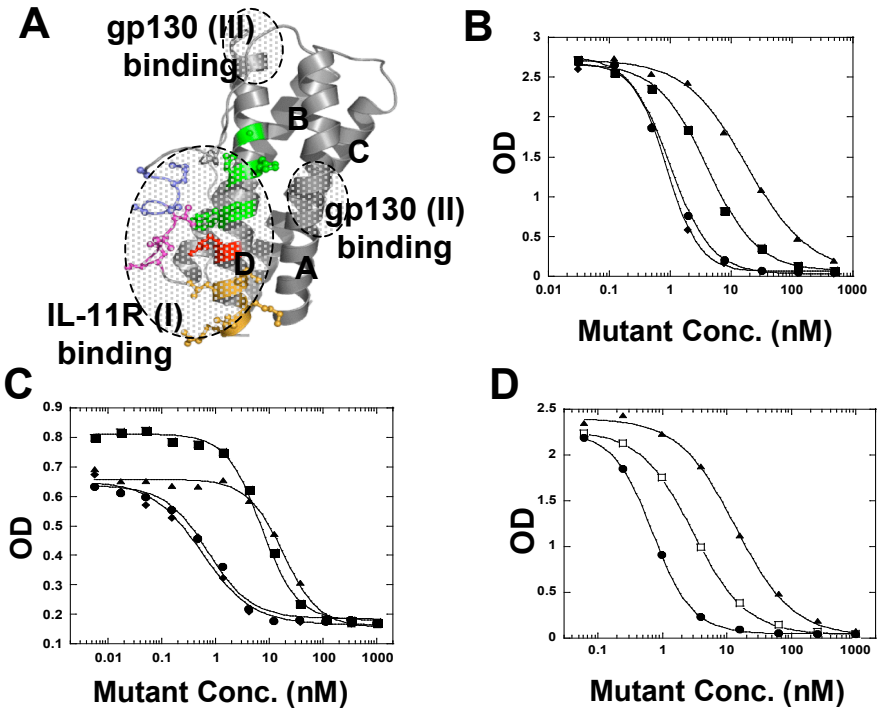

Figure E1. ***A***, a model of IL-11 generated using the Swiss model server by homology modelling on the structure of IL-6 (A). The IL-11 receptor interfaces are shaded and labelled with the gp130 (site II and site III) and IL-11Rα (site I) receptor interfaces. 4 libraries were generated: library 1 (purple) targeting residues 58-62, library 2 (pink) targeting residues 63-67 generated on a clone optimised for residues 58-62, library 3 (green) targeting residues 158, 161, 162, 165, 166 and library 4 (tan) targeting residues 172, 173, 175, 176, 177. Arginine residue R169 (red) was not randomized due to its critical role in IL-11Rα binding. ***B***, Representative clones from libraries 1, 2 and 3 were expressed as soluble protein and tested in a competition ELISA for comparison with unoptimised mIL-11 with only the W147A change (▲, EC_{50} = 18 nM), Clone 1.21-W147A was 20-fold improved (●, 58-62 = PAIDY, EC_{50} = 0.86 nM), Clone 3.5A-W147A (■) was 5-fold improved (155-168 = DGLETTLDF, EC_{50} = 3.6 nM), and Clone 2.4-W147A (♦) was 20-fold improved to similar level of clone 1.21 (58-67 = PAIDYVLNED, EC_{50} = 0.84 nM). ***C***, Representative clones from libraries 1, 2 and 3 were expressed as soluble protein and tested as antagonists in the IL-11 responsive cell line for comparison with unoptimised mIL-11 with only the W147A change (▲, EC_{50} = 19 nM), Clone 1.21-W147A was 22-fold improved (●, 58-62 = PAIDY, EC_{50} = 0.84 nM), Clone 3.5A-W147A (■) was 2-fold improved (155-168 = DGLETTLDF, EC_{50} = 8.2nM), and Clone 2.4-W147A (♦) was 30-fold improved and similar to clone 1.21 (58-67 = PAIDYVLNED, EC_{50} = 0.6 nM). ***D***, The antagonist activity of PEGylated mIL-11-W147A -Clone 1.21 (□, EC_{50} = 3.0 nM) was compared with mIL-11-W147A (▲, EC_{50} = 13 nM) and W147A-Clone 1.21 (●, EC_{50} = 0.71 nM) in the IL-11 responsive cell line.