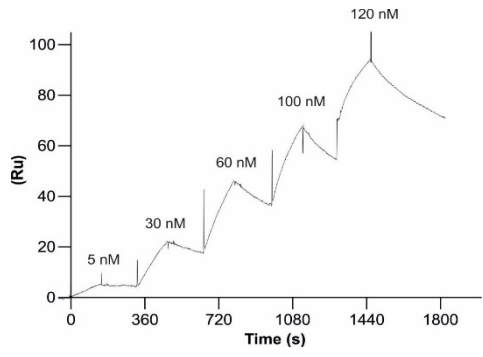


Fig. S1. *A* Quantitative SPR experiment with a CM5 chip with immobilized IL-6 and injection of sIL-6R. The gray line represents the curve of the sensorgram resulting from sIL-6R binding to IL-6. *B* IL-6-induced proliferation of Ba/F3-gp130-IL-6R cells. Half-maximal proliferation was achieved with 8.4 pM IL-6. One representative experiment out of three is shown. Error bars represent the standard deviation (SD). *C* Determination of VHH6 concentrations for an optimal stabilization of IL-6:sIL-6R complexes by ELISA. IL-6 concentrations from 5–50 ng/ml in combination with 50 ng/ml sIL-6R were used. One representative experiment out of three is shown. Error bars represent the SD. *D* Recombinant IL-6 (50 ng/ml), sIL-6R (50 ng/ml) and VHH6 (1 or 10 µg/ml) were used to analyze cross-reactivity of VHH6 for ELISA measurements.

Fig. S2. Quantitative analysis of VHH6-supported classic and trans-signaling-induced STAT3 phosphorylation. (A) Using ImageJ for quantification of Western blots from three independent experiments as shown in the lower panel of Figure 2F, our data indicate that the increase in pSTAT3 induced by VHH6 for IL-6 trans-signaling was significant. (B) Using ImageJ for quantification of Western blots from three independent experiments as shown in the lower panel of Figure 2G, our data indicate that the differences in pSTAT3 induced by VHH6 for IL-6 classic was not significant.

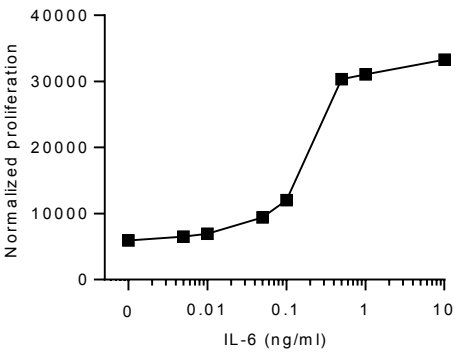
# Supplemental Figure 1

**A**

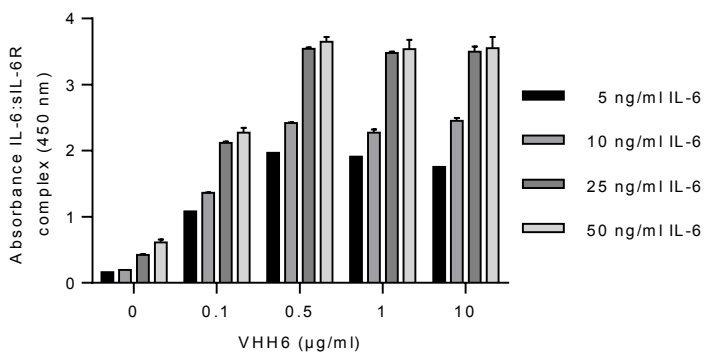


IL-6 immobilized	$K_a$ (1/M.s)	SE ( $K_a$ )	$K_{off}$ (1/s)	SE ( $K_{off}$ )	$K_d$ (M)
sIL-6R	2.77E+4	5.5E+2	6.132E-4	1.7E-5	2.210E-8

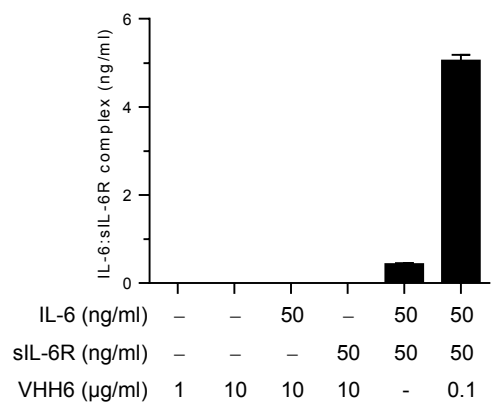
**B**



**C**



**D**



# Supplemental Figure 2

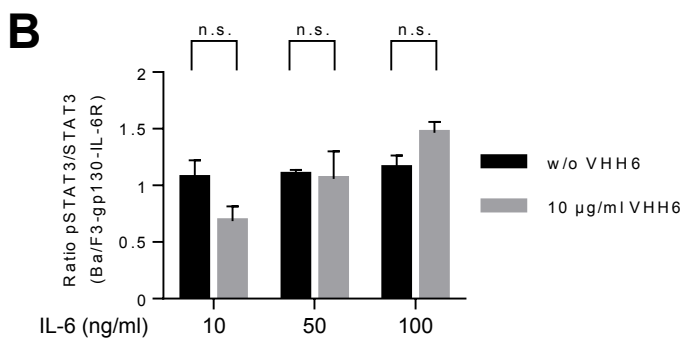
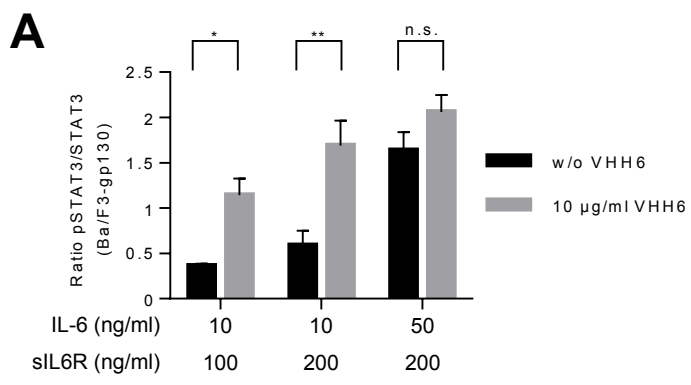


Table S1: Calculations of IL-6 and sIL-6R complex formation based on the  $K_d$  of 22 nM of (recombinant proteins).

IL-6 ng/ml	nM	sIL-6R ng/ml	nM	Free IL-6	IL-6 in complex	Free sIL-6R	sIL-6R in complex
1	0.0422	50	0.97	95.8% (0.0404 nM)	4.2% (0.0018 nM)	99.8% (0.968 nM)	0.2 % (0.002 nM)
10	0.422	50	0.97	95.8% (0.404 nM)	4.2% (0.018 nM)	98.2% (0.952 nM)	1.8% (0.018 nM)
50	2.11	50	0.97	96.1% (2.03 nM)	3.9% (0.8 nM)	91.6% (0.888 nM)	8.4% (0.082 nM)
1000	42.2	50	0.97	98.5% (41.5 nM)	1.5% (0.7 nM)	34.6% (0.336 nM)	65.4% (0.634 nM)

Table S2: Summary of all IL-6:sIL-6R complex formation experiments from Figure 1B and 3C with three concentrations of recombinant sIL-6R and endogenous sIL-6R combined with increasing amounts of IL-6 quantified by IL-6:sIL-6R complex ELISA.

IL-6 (ng/ml)	Recombinant sIL-6R (25 ng/ml)			Recombinant sIL-6R (50 ng/ml)			Recombinant sIL-6R (75 ng/ml)			Endogenous sIL-6R (52.9 ± 16.3 ng/ml)		
	IL-6:sIL-6R complex (ng/ml)	Bound IL-6 (%)	Bound sIL-6R (%)	IL-6:sIL-6R complex (ng/ml)	Bound IL-6 (%)	Bound sIL-6R (%)	IL-6:sIL-6R complex (ng/ml)	Bound IL-6 (%)	Bound sIL-6R (%)	IL-6:sIL-6R complex (ng/ml)	Bound IL-6 (%)	Bound sIL-6R (%)
1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.16 (± 0.27)	5.32 (± 8.7)	0.27 (± 0.48)
5	0.06 (± 0.03)	0.42 (± 0.22)	0.18 (± 0.09)	0.07 (± 0.07)	0.49 (± 0.48)	0.1 (± 0.1)	0.02 (± 0.04)	0.13 (± 0.25)	0.02 (± 0.03)	0.26 (± 0.39)	1.64 (± 2.4)	0.4 (± 0.65)
10	0.18 (± 0.01)	0.59 (± 0.04)	0.5 (± 0.03)	0.35 (± 0.08)	1.09 (± 0.26)	0.47 (± 0.11)	0.37 (± 0.08)	1.17 (± 0.27)	0.33 (± 0.07)	0.54 (± 0.49)	1.71 (± 1.5)	0.81 (± 0.81)
25	0.69 (± 0.05)	0.88 (± 0.07)	1.9 (± 0.15)	1.18 (± 0.22)	1.48 (± 0.28)	1.61 (± 0.3)	1.4 (± 0.23)	1.77 (± 0.3)	1.28 (± 0.21)	1.59 (± 0.63)	2.01 (± 0.8)	2.24 (± 0.87)
50	1.3 (± 0.008)	0.82 (± 0.01)	3.56 (± 0.02)	2.37 (± 0.44)	1.49 (± 0.28)	3.24 (± 0.59)	2.68 (± 0.3)	1.69 (± 0.19)	2.45 (± 0.27)	2.99 (± 1.05)	1.89 (± 0.67)	4.14 (± 1.09)
100	2.3 (± 0.13)	0.73 (± 0.04)	6.34 (± 0.36)	4.05 (± 0.86)	1.28 (± 0.27)	5.55 (± 1.17)	5.05 (± 0.42)	1.59 (± 0.13)	4.61 (± 0.36)	5 (± 1.85)	1.58 (± 0.58)	6.82 (± 1.48)
200	3.3 (± 0.04)	0.52 (± 0.01)	9.12 (± 0.12)	5.56 (± 1.22)	0.88 (± 0.19)	7.61 (± 1.67)	6.42 (± 0.88)	1.01 (± 0.14)	5.86 (± 0.8)	7.55 (± 2.67)	1.19 (± 0.42)	10.35 (± 2.22)
1000	5 (± 0.28)	0.16 (± 0.01)	13.78 (± 0.79)	8.49 (± 2.1)	0.27 (± 0.07)	11.6 (± 2.88)	9.86 (± 0.74)	0.31 (± 0.02)	9 (± 0.6)	11.3 (± 4.71)	0.36 (± 0.15)	15.24 (± 3.54)

Table S3: Overview of IL-6:sIL-6R complex formation based on the  $K_d$  calculation (22 nM) compared with ELISA measurements from Figure 1B and 3C.

Titration	Recombinant sIL-6R (25 ng/ml)		Recombinant sIL-6R (50 ng/ml)		Recombinant sIL-6R (75 ng/ml)		Endogenous sIL-6R (52.9 ± 16.3 ng/ml)	
	$K_d$ calculation (bound IL-6, %)	ELISA (bound IL-6, %)	$K_d$ calculation (bound IL-6, %)	ELISA (bound IL-6, %)	$K_d$ calculation (bound IL-6, %)	ELISA (bound IL-6, %)	$K_d$ calculation (bound IL-6, %)	ELISA (bound IL-6, %)
1	2.2	n.d.	4.2	n.d.	6.2	n.d.	4.5	5.32 (± 8.7)
5	2.1	0.42 (± 0.22)	4.2	0.49 (± 0.48)	6.2	0.13 (± 0.25)	4.4	1.64 (± 2.4)
10	2.1	0.59 (± 0.04)	4.2	1.09 (± 0.26)	6.1	1.17 (± 0.27)	4.4	1.71 (± 1.5)
25	2.1	0.88 (± 0.07)	4	1.48 (± 0.28)	6	1.77 (± 0.3)	4.3	2.01 (± 0.8)
50	2	0.82 (± 0.01)	3.9	1.49 (± 0.28)	4.7	1.69 (± 0.19)	4.1	1.89 (± 0.67)
100	1.8	0.73 (± 0.04)	3.6	1.28 (± 0.27)	5.3	1.59 (± 0.13)	3.8	1.58 (± 0.58)
200	1.6	0.52 (± 0.01)	3.1	0.88 (± 0.19)	4.6	1.01 (± 0.14)	3.3	1.19 (± 0.42)
1000	0.8	0.16 (± 0.01)	1.5	0.27 (± 0.07)	2.2	0.31 (± 0.02)	1.6	0.36 (± 0.15)

Table S4: Summary of all IL-6:sIL-6R complex formation experiments from Figure 1D with three concentrations of IL-6 combined with increasing amounts of sIL-6R quantified by IL-6:sIL-6R complex ELISA.

sIL-6R (ng/ml)	Recombinant IL-6 (1 ng/ml)			Recombinant IL-6 (50 ng/ml)			Recombinant IL-6 (100 ng/ml)		
	IL-6:sIL-6R complex (ng/ml)	Bound IL-6 (%)	Bound sIL-6R (%)	IL-6:sIL-6R complex (ng/ml)	Bound IL-6 (%)	Bound sIL-6R (%)	IL-6:sIL-6R complex (ng/ml)	Bound IL-6 (%)	Bound sIL-6R (%)
1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.04 ( $\pm$ 0.09)	0.01 ( $\pm$ 0.02)	2.83 ( $\pm$ 5.98)
5	n.d.	n.d.	n.d.	0.01 ( $\pm$ 0.01)	0.035 ( $\pm$ 0.028)	0.15 ( $\pm$ 0.12)	0.37 ( $\pm$ 0.14)	0.11 ( $\pm$ 0.04)	5 ( $\pm$ 1.87)
10	n.d.	n.d.	n.d.	0.07 ( $\pm$ 0.06)	0.21 ( $\pm$ 0.17)	0.46 ( $\pm$ 0.38)	0.87 ( $\pm$ 0.26)	0.27 ( $\pm$ 0.08)	5.95 ( $\pm$ 1.8)
25	n.d.	n.d.	n.d.	0.24 ( $\pm$ 0.07)	0.77 ( $\pm$ 0.21)	0.67 ( $\pm$ 0.18)	2.59 ( $\pm$ 0.64)	0.81 ( $\pm$ 0.2)	7.08 ( $\pm$ 1.77)
50	0.01 ( $\pm$ 0.01)	0.22 ( $\pm$ 0.19)	0.009 ( $\pm$ 0.008)	0.44 ( $\pm$ 0.07)	1.38 ( $\pm$ 0.21)	0.6 ( $\pm$ 0.09)	4.85 ( $\pm$ 1.09)	1.52 ( $\pm$ 0.34)	6.65 ( $\pm$ 1.5)
100	0.01 ( $\pm$ 0.01)	0.43 ( $\pm$ 0.35)	0.009 ( $\pm$ 0.007)	0.74 ( $\pm$ 0.07)	2.31 ( $\pm$ 0.21)	0.5 ( $\pm$ 0.04)	7.65 ( $\pm$ 1.23)	2.4 ( $\pm$ 0.38)	5.24 ( $\pm$ 0.84)
200	0.02 ( $\pm$ 0.01)	0.54 ( $\pm$ 0.42)	0.006 ( $\pm$ 0.004)	0.91 ( $\pm$ 0.15)	2.87 ( $\pm$ 0.47)	0.31 ( $\pm$ 0.05)	11.38 ( $\pm$ 1.87)	3.58 ( $\pm$ 0.59)	3.9 ( $\pm$ 0.64)
1000	0.003 ( $\pm$ 0.01)	0.09 ( $\pm$ 0.23)	0.0002 ( $\pm$ 0.005)	0.55 ( $\pm$ 0.16)	1.73 ( $\pm$ 0.5)	0.038 ( $\pm$ 0.011)	13.51 ( $\pm$ 4.12)	4.25 ( $\pm$ 1.29)	0.93 ( $\pm$ 0.28)

Table S5: Overview of IL-6:sIL-6R complex formation based on the  $K_d$  calculation (22 nM) compared with ELISA measurements from Figure 1D.

Titration	Recombinant IL-6 (1 ng/ml)		Recombinant IL-6 (50 ng/ml)		Recombinant IL-6 (100 ng/ml)	
	$K_d$ calculation (bound sIL-6R, %)	ELISA (bound sIL-6R, %)	$K_d$ calculation (bound sIL-6R, %)	ELISA (bound sIL-6R, %)	$K_d$ calculation (bound sIL-6R, %)	ELISA (bound sIL-6R, %)
1	0.2	n.d.	1.9	n.d.	16.1	2.83 ( $\pm$ 5.98)
5	0.2	n.d.	1.9	0.15 ( $\pm$ 0.12)	16	5 ( $\pm$ 1.87)
10	0.2	n.d.	1.9	0.46 ( $\pm$ 0.38)	16	5.95 ( $\pm$ 1.8)
25	0.2	n.d.	1.8	0.67 ( $\pm$ 0.18)	15.8	7.08 ( $\pm$ 1.77)
50	0.2	0.009 ( $\pm$ 0.008)	1.8	0.6 ( $\pm$ 0.09)	15.6	6.65 ( $\pm$ 1.5)
100	0.2	0.009 ( $\pm$ 0.007)	1.7	0.5 ( $\pm$ 0.04)	15.1	5.24 ( $\pm$ 0.84)
200	0.2	0.006 ( $\pm$ 0.004)	1.6	0.31 ( $\pm$ 0.05)	14.3	3.9 ( $\pm$ 0.64)
1000	0.1	0.0002 ( $\pm$ 0.005)	1	0.038 ( $\pm$ 0.011)	9.6	0.93 ( $\pm$ 0.28)

Table S6: Overview of IL-6-sIL-6R complex formation based on the  $K_d$  calculation (22 nM) compared with ELISA measurements from Figure 1D.

Titration	Recombinant IL-6 (1 ng/ml)		Recombinant IL-6 (50 ng/ml)		Recombinant IL-6 (100 ng/ml)	
	$K_d$ calculation (bound IL-6, %)	ELISA (bound IL-6, %)	$K_d$ calculation (bound IL-6, %)	ELISA (bound IL-6, %)	$K_d$ calculation (bound IL-6, %)	ELISA (bound IL-6, %)
1	0.1	n.d.	0.1	n.d.	0.1	0.01 ( $\pm$ 0.02)
5	0.4	n.d.	0.4	0.035 ( $\pm$ 0.028)	0.4	0.11 ( $\pm$ 0.04)
10	0.9	n.d.	0.9	0.21 ( $\pm$ 0.17)	0.7	0.27 ( $\pm$ 0.08)
25	2.2	n.d.	2.1	0.77 ( $\pm$ 0.21)	1.8	0.81 ( $\pm$ 0.2)
50	4.2	0.22 ( $\pm$ 0.19)	4.2	1.38 ( $\pm$ 0.21)	3.6	1.52 ( $\pm$ 0.34)
100	8.1	0.43 ( $\pm$ 0.35)	8	2.31 ( $\pm$ 0.21)	7	2.4 ( $\pm$ 0.38)
200	15	0.54 ( $\pm$ 0.42)	14.8	2.87 ( $\pm$ 0.47)	13.1	3.58 ( $\pm$ 0.59)
1000	46.8	0.09 ( $\pm$ 0.23)	46.6	1.73 ( $\pm$ 0.5)	44.4	4.25 ( $\pm$ 1.29)



Table S7: Overview IL-6:sIL-6R complex formation without (w/o) or with VHH6 from Figure 2B and 3E.

IL-6 (ng/ml)	Recombinant sIL-6R			Endogenous sIL-6R			
	sIL-6R (ng/ml)	IL-6:sIL-6R complex (w/o VHH6, ng/ml)	IL-6:sIL-6R complex (with VHH6, ng/ml)	IL-6 (ng/ml)	sIL-6R (ng/ml)	IL-6:sIL-6R complex (w/o VHH6, ng/ml)	IL-6:sIL-6R complex (with VHH6, ng/ml)
0.1	50	n.d.	0.12 ( $\pm$ 0.1)	0.1	54 ( $\pm$ 16)	n.d.	0.2 ( $\pm$ 0.32)
0.25	50	n.d.	0.37 ( $\pm$ 0.15)	0.25	54 ( $\pm$ 16)	n.d.	0.78 ( $\pm$ 0.27)
0.5	50	n.d.	0.79 ( $\pm$ 0.25)	0.5	54 ( $\pm$ 16)	n.d.	2 ( $\pm$ 0.31)
1	50	n.d.	1.18 ( $\pm$ 0.2)	1	54 ( $\pm$ 16)	n.d.	4.22 ( $\pm$ 0.37)

Table S8: IL-6:sIL-6R complex measurements in sgp130-containing and -depleted serum from Figure 4E.

Titration	Sgp130-containing serum (388 ng/ml)		Sgp130-depleted serum (7.5 ng/ml)	
	IL-6:sIL-6R complex (ng/ml)	Bound sIL-6R (%) (40.5 ng/ml sIL-6R)	IL-6:sIL-6R complex (ng/ml)	Bound sIL-6R (%) (39.6 ng/ml sIL-6R)
50	3 ( $\pm$ 0.4)	5.18 ( $\pm$ 0.68)	3.0 ( $\pm$ 0.3)	5.27 ( $\pm$ 0.53)
100	4.88 ( $\pm$ 0.43)	8.26 ( $\pm$ 0.73)	5.3 ( $\pm$ 0.34)	9.3 ( $\pm$ 0.6)
200	6.83 ( $\pm$ 0.73)	11.56 ( $\pm$ 1.24)	7.99 ( $\pm$ 0.81)	13.83 ( $\pm$ 1.4)
1000	11.12 ( $\pm$ 0.94)	18.81 ( $\pm$ 1.6)	13.4 ( $\pm$ 1.46)	23.24 ( $\pm$ 2.54)