ONLINE SUPPLEMENTAL MATERIAL

Legends to Supplemental Figures

FIG. S1. **Insulin and IGF-II at equimolar concentration differ in their ability to promote IR-A internalization.** The level of IR-A internalization in R-/IR-A cells was determined by ELISA at different time points after insulin (INS) and IGF-II stimulation, as described in Experimental Procedures. Ligands were used at 30 nM. Data are the averages ± SD of three independent experiments. Statistical significance was determined using Student's t test for repeated measures, **p* <0.05; ****p* <0.01 (INS or IGF-II vs SFM) and using ANOVA with Bonferroni's multiple-comparison test, *p* <0.01; (INS vs IGF-II).

FIG. S2. **An insulin analog with lower affinity than insulin for the IR-A induces lower levels of IR-A phosphorylation and signaling**. The insulin analog NMeTyr^{B26}-insulin has been previously described (22). (A) Inhibition of binding of human ^{125}I-insulin to the insulin receptor (IR-A isoform) in the membranes of IM-9 lymphocytes by human insulin (●), NMeTyr^{B26}-insulin analog (▲) and human IGF-II (■). Binding was performed as described by Gauguin at al. (23). [a]K_d represents dissociation constant of binding of insulin and insulin analog to the IR. Each value represents the mean ± the SD of the mean of multiple determinations (n). [b]Relative binding affinity (Rel K_d) defined as (K_d of human insulin/Kd of analog) x 100. (B) Serum-starved R-/IR-A cells were stimulated with either insulin, IGF-II or NMeTyr^{B26}-insulin at 1, 5 and 30 nM for the indicated time points. IR-A tyrosine-phosphorylation was determined by immunoblot as described in Experimental Procedures. The experiment shown is representative of two independent experiments. (C) Akt and ERK1/2 activation was assessed by immunoblot with anti-phosphospecific antibodies at different time points of ligand stimulation as described above. Blots are representative of two independent experiments.

FIG. S3. **Effect of physiological concentration of ligands on IR-A and IRS-1 stability**. IR-A and IRS-1 levels in R-/IR-A cells were determined by immunoblot after stimulation with 5 nM of either insulin, IGF-II or NMeTyr^{B26}-insulin. The total amount of protein loaded on the gel was monitored using anti-β-actin polyclonal antibodies. Quantification was performed by densitometry using NIH ImageJ software. The data are presented as means ± SD. Statistical significance was determined using two-way ANOVA with Bonferroni's multiple-comparison test. ****p* <0.001; ***p* <0.01; **p* <0.05.

FIG. S4**. Insulin and IGF-II differentially affect IR-A internalization of NIH3T3/IR-A, R+A10 and MDA-MB-157 breast cancer cells**. (A, B, C) Protein levels for the IR-A and IGF-IR were determined by immunoblot analysis with specific polyclonal antibodies, as described in Experimental Procedures. (A, B, C) The level of cell surface IR-A in the various cell lines was determined by ELISA at different time points of insulin (INS) and IGF-II stimulation, as described in Experimental Procedures. Data are the averages ± SD of three independent experiments. Statistical significance was determined using Student's t test for repeated measures, **p* <0.05 (INS vs IGF-II). (A, B) IR and IRS-1 levels were assessed as described in Experimental Procedures. Blots are representative of three independent experiments.

Figure S1, Morcavallo et al.

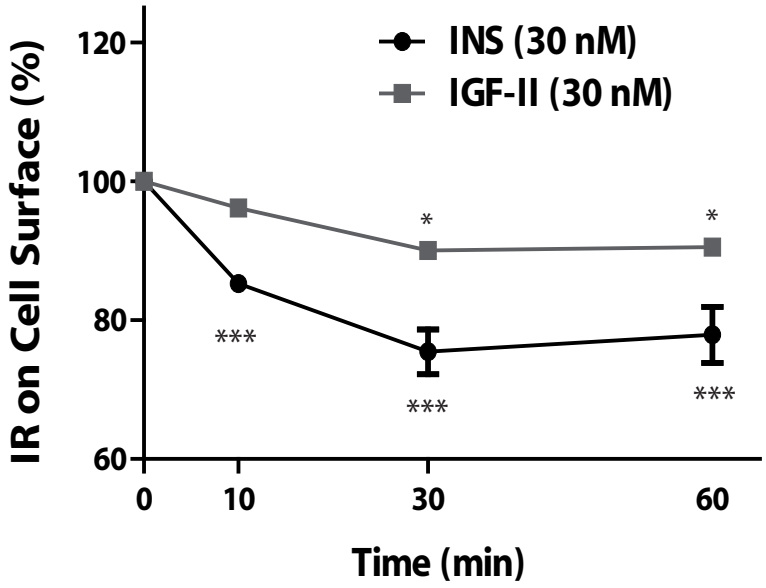
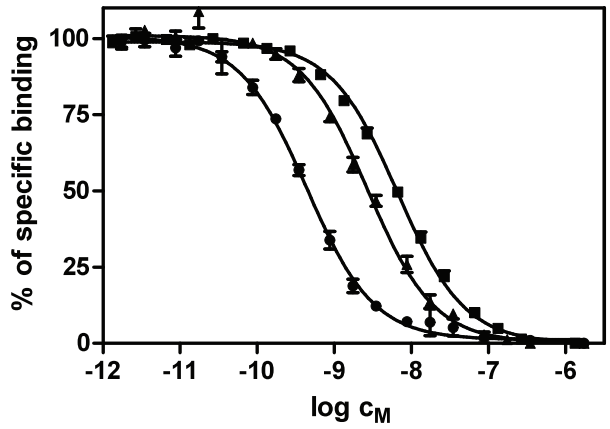


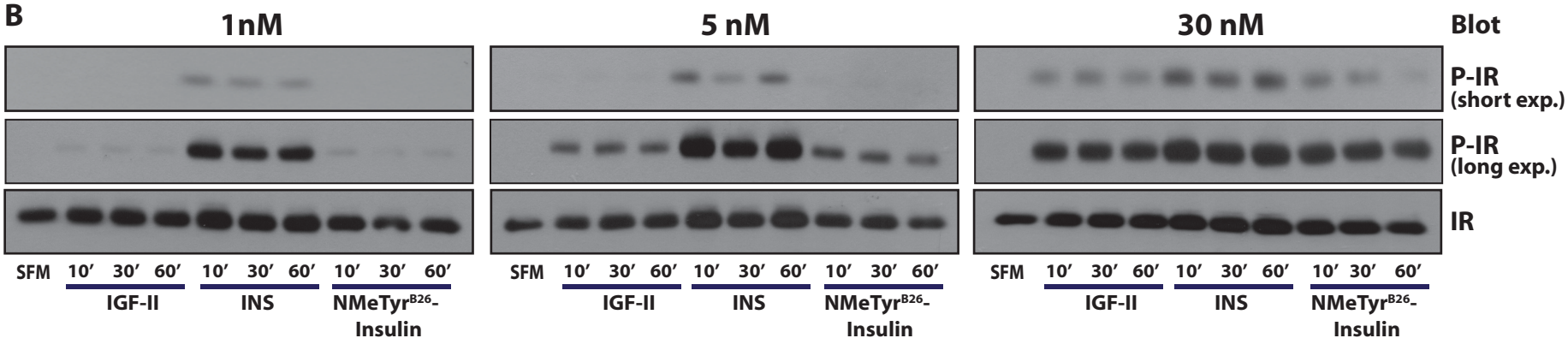
Figure S2, Morcavallo et al.

A



	K_d ± S.D. (nM)	Rel K_d (%) ± S.D.
Human insulin	0.27 ± 0.04	100
NMeTyrB^{26}-insulin	1.68 ± 0.27	16.1 ± 2.6
IGF-II	3.96 ± 0.56	6.8 ± 1.0

B



C

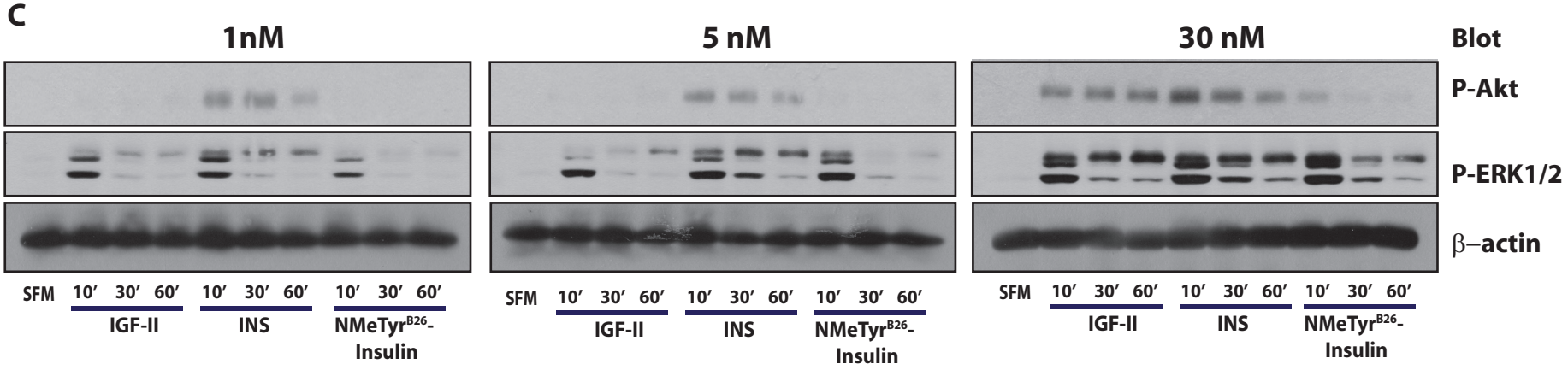


Figure S3, Morcavallo et al.

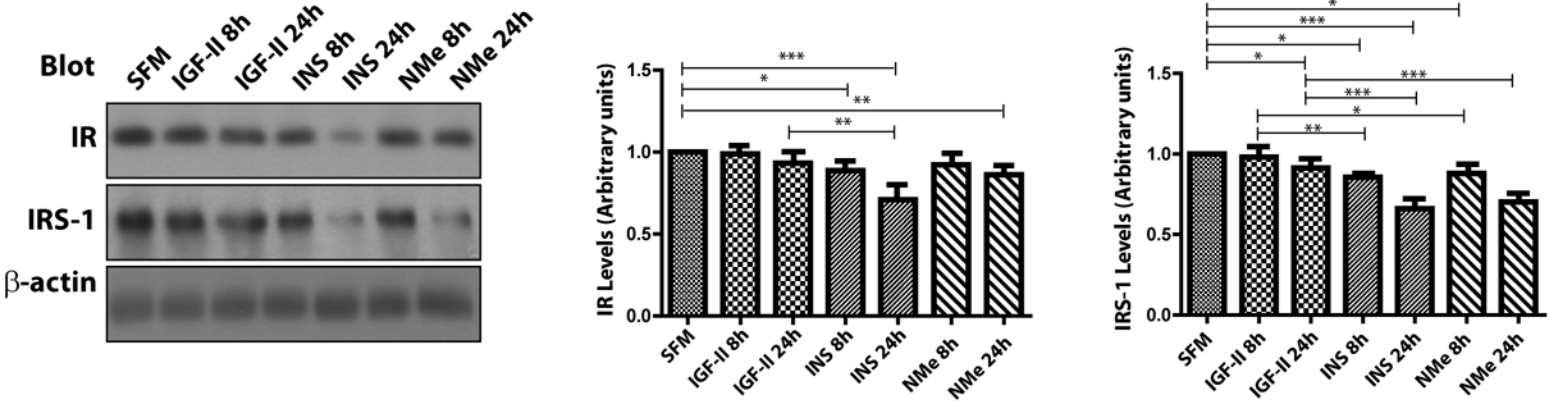


Figure S4, Morcavallo et al.