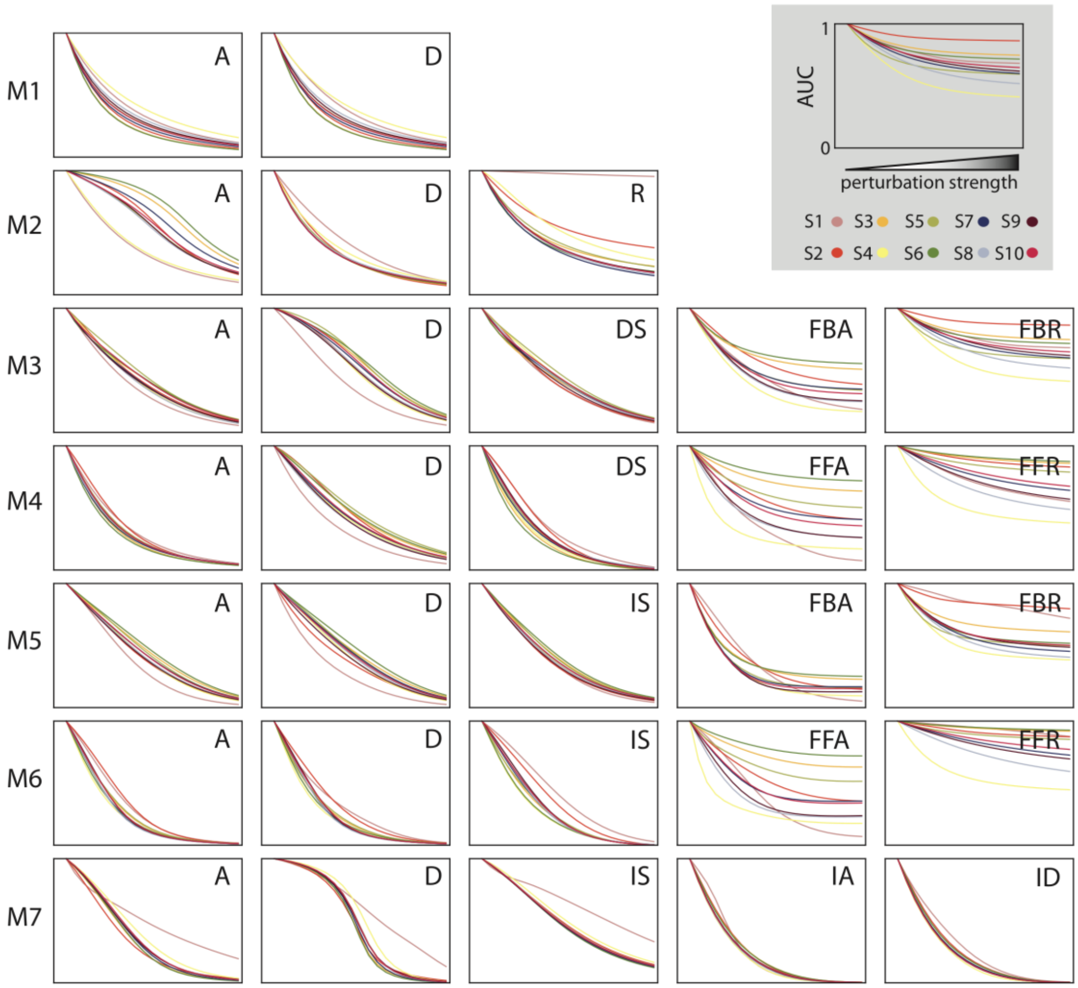
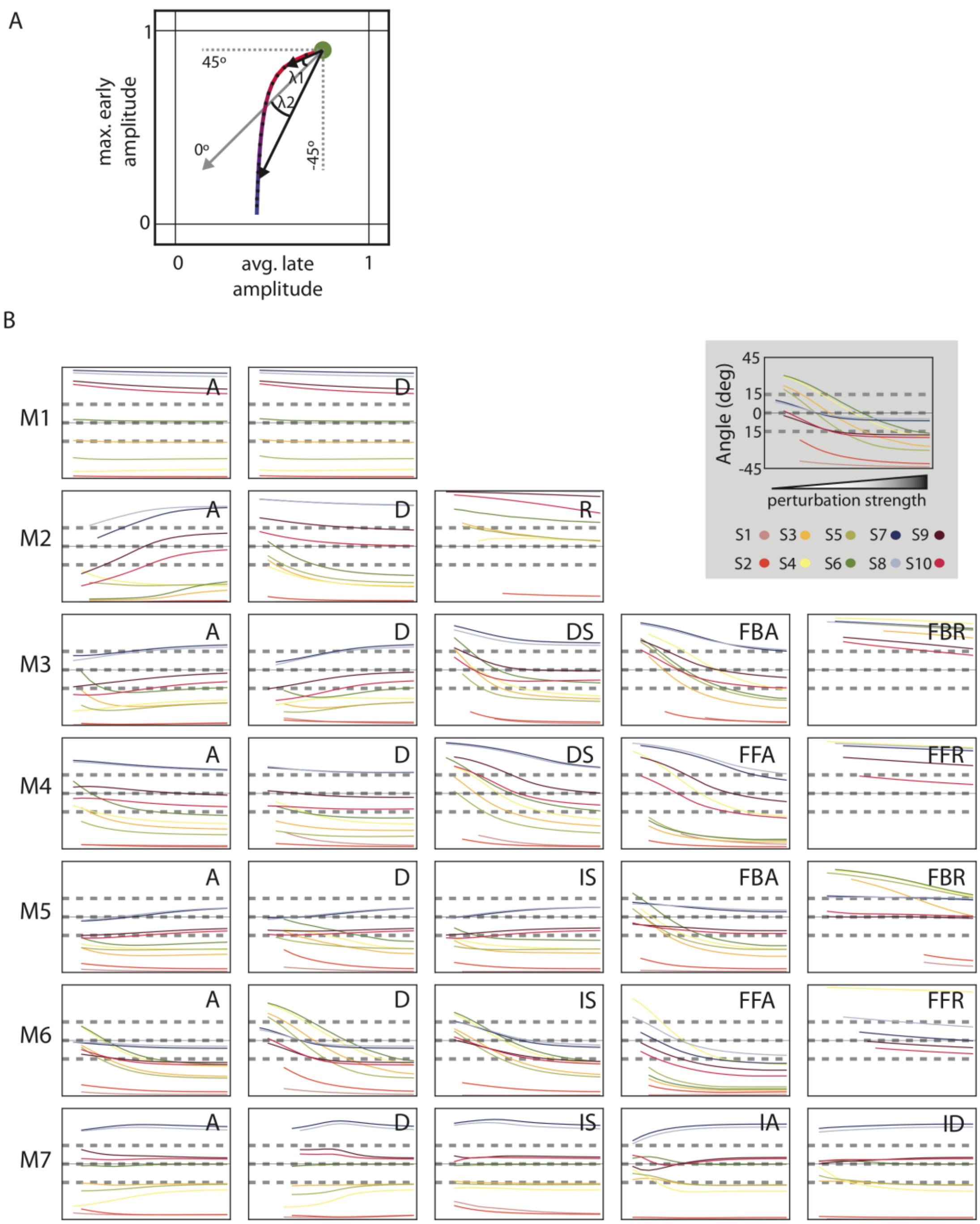


Supplemental Figure S1 (relate to Figure 2B). Area under the time course curves as a function of the perturbation strength normalized to the unperturbed value (leftmost point).



Supplemental Figure S2 (relate to Figure 3). (A) Definition of the angle criteria for determing early or late selectivity. (B) Early or late selectivity as a function of the perturbation strength. The unperturbed state is on the left of the axis. Lines starting mid-axis arise because the effect of the perturbatio is small and remains below the threshold (see methods).

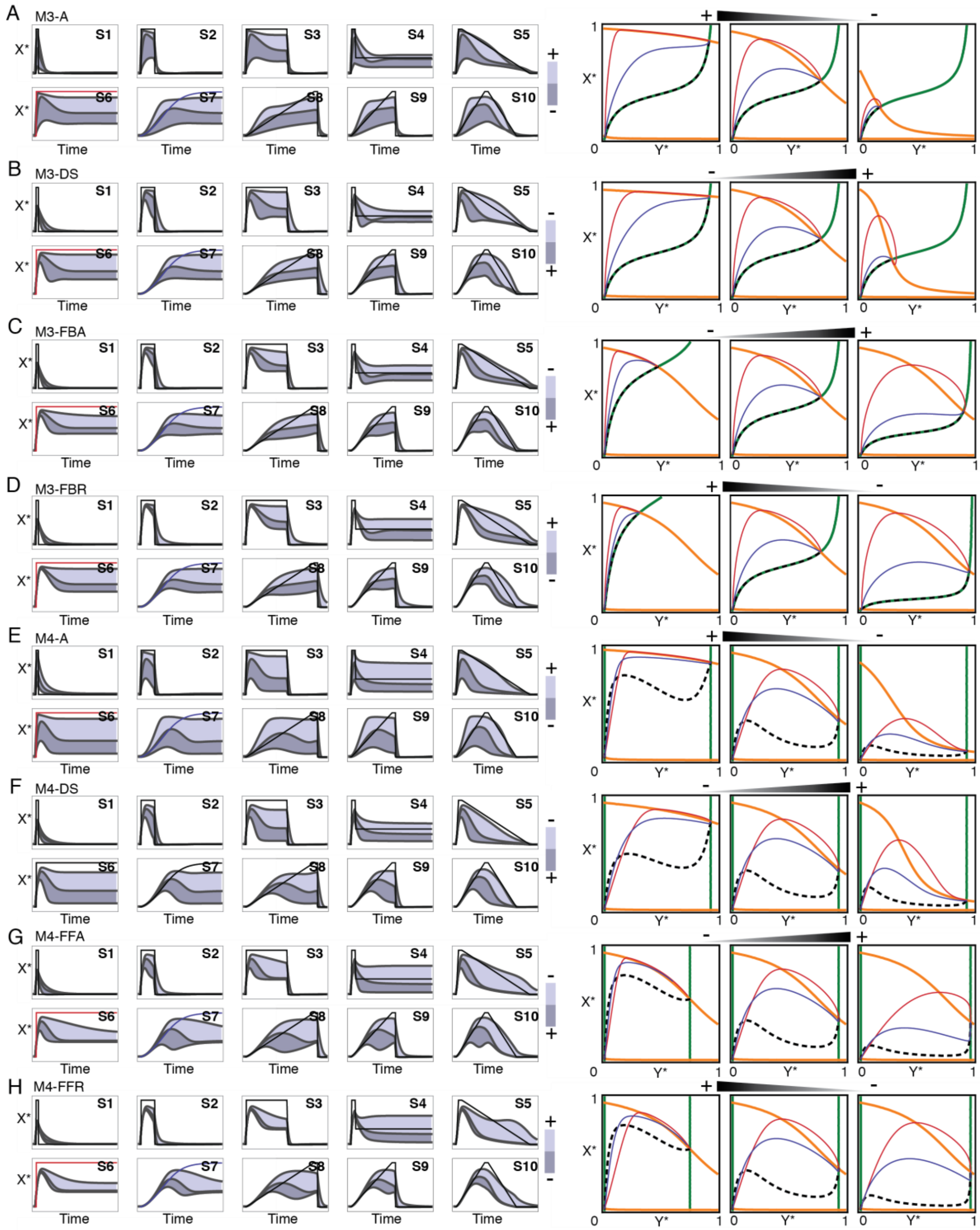
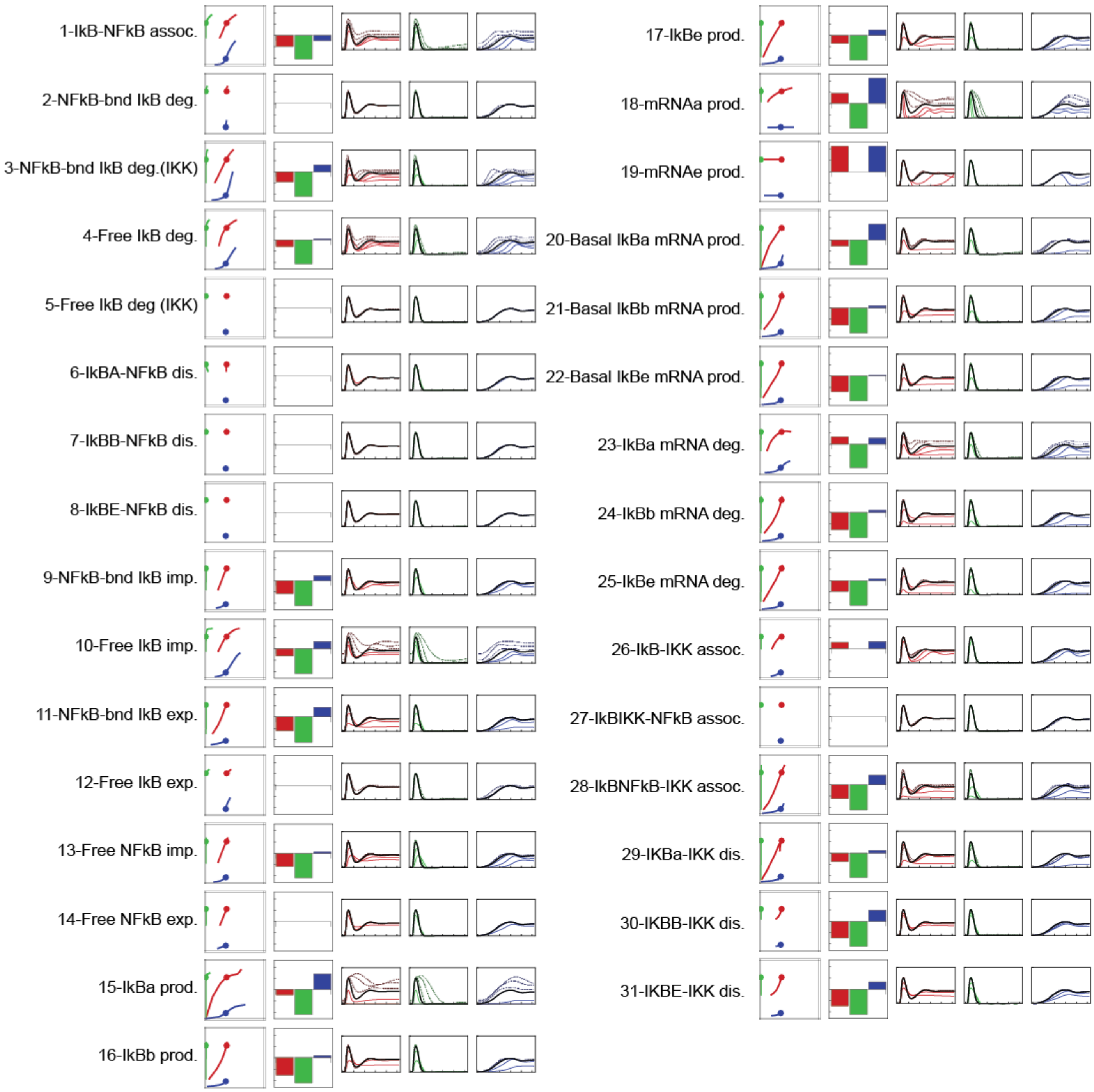
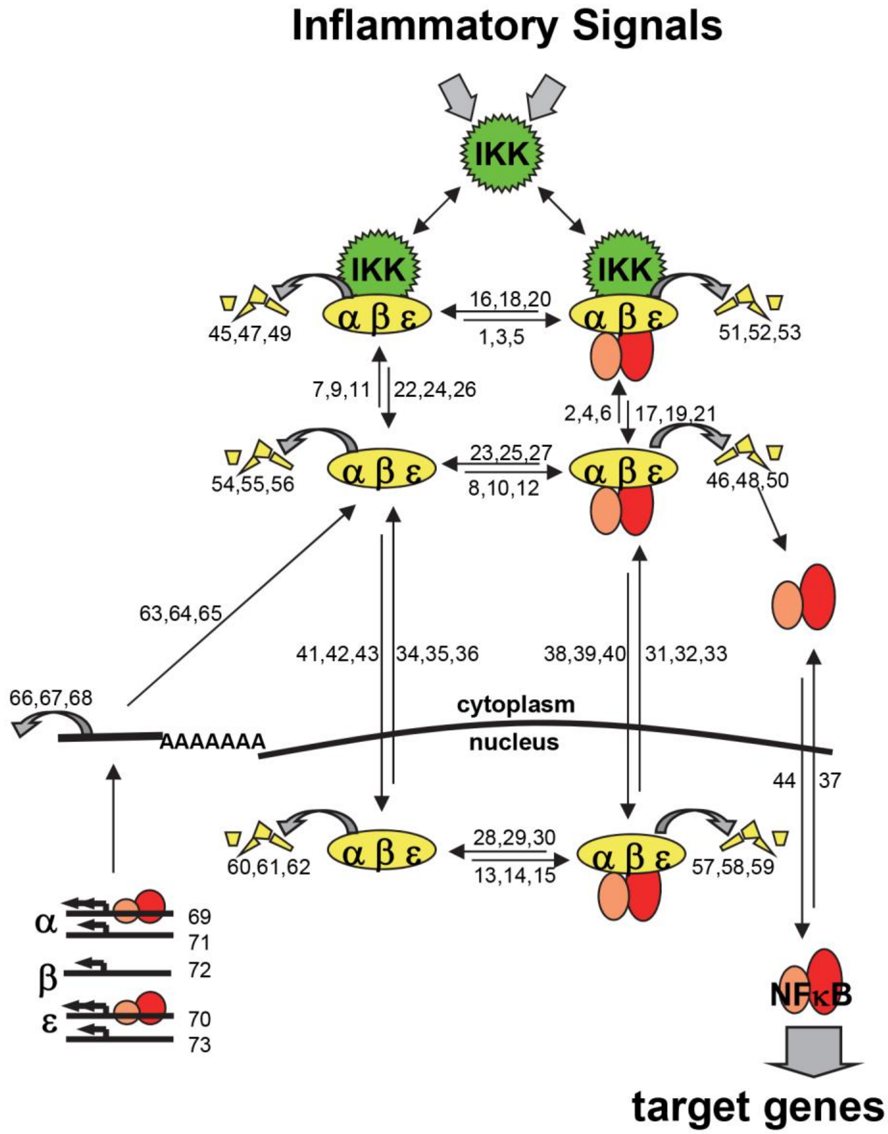


Figure S3. Detailed Analysis of modules M3 and M4 (relate to Figure 4): Time courses and projections of the phase space for modules M3 and M4. Color coding similar to Figure 4. (A) Perturbation A in M3 distorts the surface of Y*. When in the unperturbed case Y* feedback is saturated, the perturbation largely affects the steady state and hence the late-term response to sustained input signals. In the region in which Y* is responsive to X*, the perturbation will affect the transient and the effect on the steady-state will depend on the steepness of the Y curve. If Y* activation is ultrasensitive, the Y* curve is virtually horizontal in that region and the perturbation does not affect the steady state. Strong perturbations (or if the feedback is strong) causing the module to operate near the left axis affect the response globally. Shifts in the shape of the X* surface will affect signal duration when the post-activation attenuation evolves over that surface. (B) Perturbation DS (strength of the deactivator) has a similar effect. In contrast with perturbation A that equalizes internal time scales and slows the kinetics of the module relative to the input, DS only equalizes internal time scales (output inhibition corresponds to paramter increase) and therefore it is less effective at reducing out-of-equilibrium signals. Because the projection of the quasi-equilibrium curve has not shifted (Y* curve is independent of S) the amplitude of strict quasi-equilibrium responses are not affected but responses may be delayed. Perturbations A and DS affect signals that relay heavily on strong out-of-equilibrium components (S1, S2, S4) or consist mostly of quasi-equlibrium phases (S7-9). (C) Feedback activation and (D) deactivation (FBA and FBR) shift the Y* curve vertically. If Y* is saturated in the unperturbed case (curves intersect on the right axis) the perturbations affect the transient phase. Because of the shifted quasi-equilibrium curve, the dynamics of the response to gradual inputs is affected, even if the steady-state level remains unchanged. For modules operating in the plateau region, the perturbation selectively affects steady state. FBA equalizes time scales and thus affects out-of-equilibrium dynamics as well. With the parameters used this effect is of little importance for sudden signals (S1-S5) but has a significant impact on gradual signals with mild out-of-equilibrium components. (E) Both, X* and Y* surfaces in M4 depend on S, but Y* surface is independent of X*. Perturbation A distorts the X* surface affecting steady-state. In general, a perturbation strong enough to affect early peaks will also suppress the steady state. (F) Similar observations hold for DS. The perturbations distort the quasi-equilibrium curves and therefore quasi-equilibrium dynamics will be affected in both timing and amplitude. Because the dose used was insuficient to suppress the early peak (higher doses would cause global suppression), this perturbations appear to primarily affect inputs with sustained components more than brief inputs such as S1-2. Notice that in this module, Y*-independent deactivation retains some specificity for out-of-equilibrium signal components because of its effect on the region of the dose response surface (low Y*) that limits the transient amplitude. Perturbations FFA (G) and FFR (H) shift the Y surface EC_{50} value respect to S and suppress steady-state before affecting the out-of-equilibrium peak (see specificity switch in Figure 3). The effect is limited by the potency of Y*; in the example, maximum activation of Y* is insufficient to fully suppress X* for high values of S, a limitation that FFA/FFR cannot overcome. These perturbations also distort the quasi-equilibrium curve and quasi-equilibrium responses to gradual input signals (S7-10). Because FFA accelerates Y* activation, this perturbation is more effective. Feed-forward based modules can produce secondary peaks upon signal termination when the EC_{50} values of X and Y are different and Y* and X* deactivation occur on comparable time scales.



Supplemental Figure S4 (relate to Figure 5): Perturbations of the NFκB network. Effect of perturbing the indicated parameter group for TNFc (red), TNFp (green), and LPS (blue) stimulation. From left to right: early (t<60') and late (120'<t<300') parts of the NFκB response (the dot represents unperturbed response, the solid and dashed branches represent inhibitory and enhancing perturbations respectively), angle in E-L space for the inhibitory branch, and timecourses (unperturbed in black). Axes as in Figure 5 (manin text).



Supplemental Figure S5 (relate to Methods): Wiring diagram for the NFκB model:
(A) Wiring diagram of the NFκB model which calculates nuclear NFκB DNA binding activity as a function of IKK activity. There are three IκB proteins that control NFκB activity. All three have the same topology but kinetic parameter values differ. Numbers correspond to the parameter IDs in tables S5 and S7.