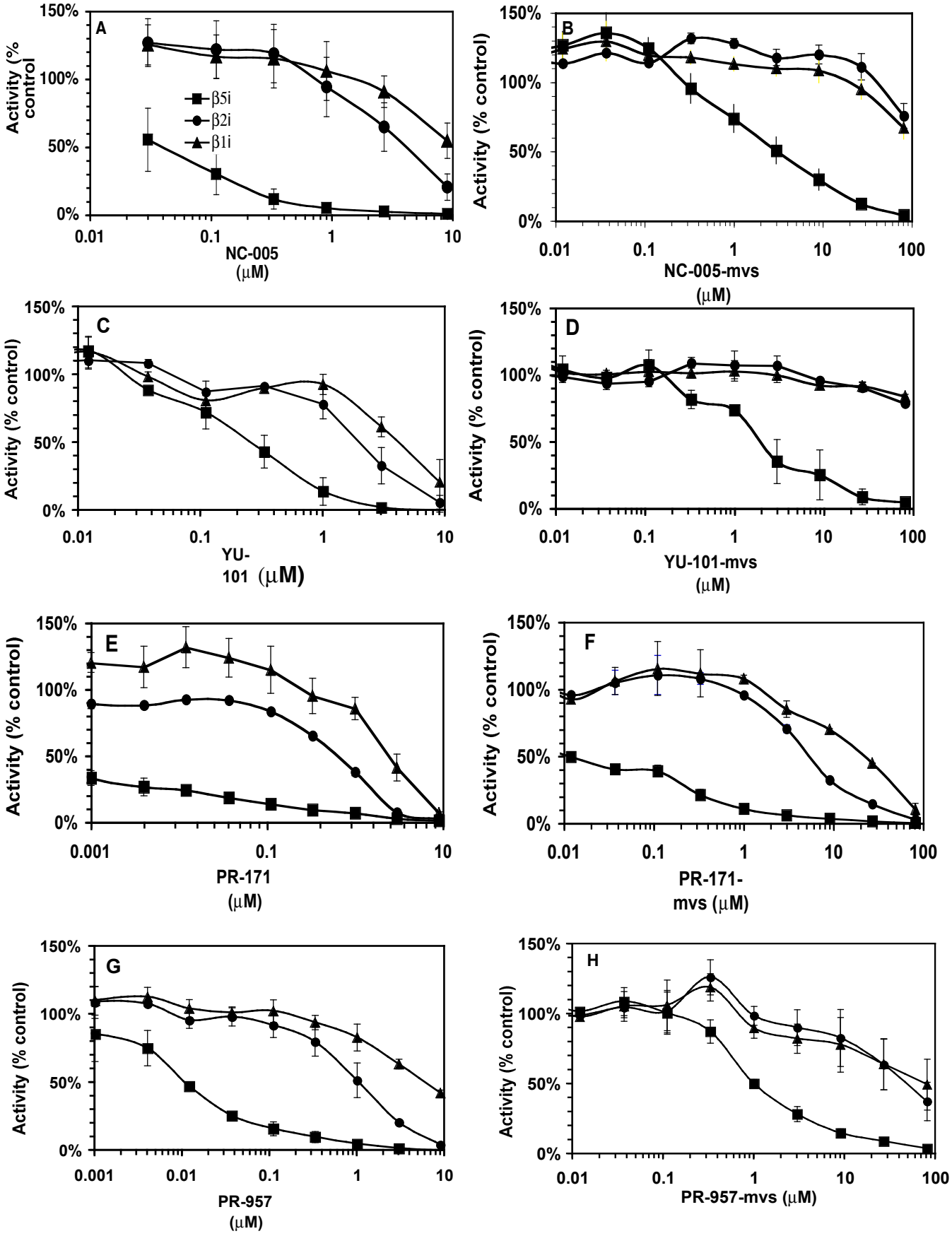

Figure S1. Inhibition of purified 26S proteasomes from rabbit spleens by epoxyketones targeting β5 and β5i sites and their methyl vinyl sulfone derivative. Squares, β5i activity; triangles, β1i activity; circles, β2i activity. On all graphs, values are averages ± S.E. of 2 or 3 independent measurements. Proteasomes were treated with inhibitors for 30 minutes followed by measurements of activities as described in Experimental Procedures.

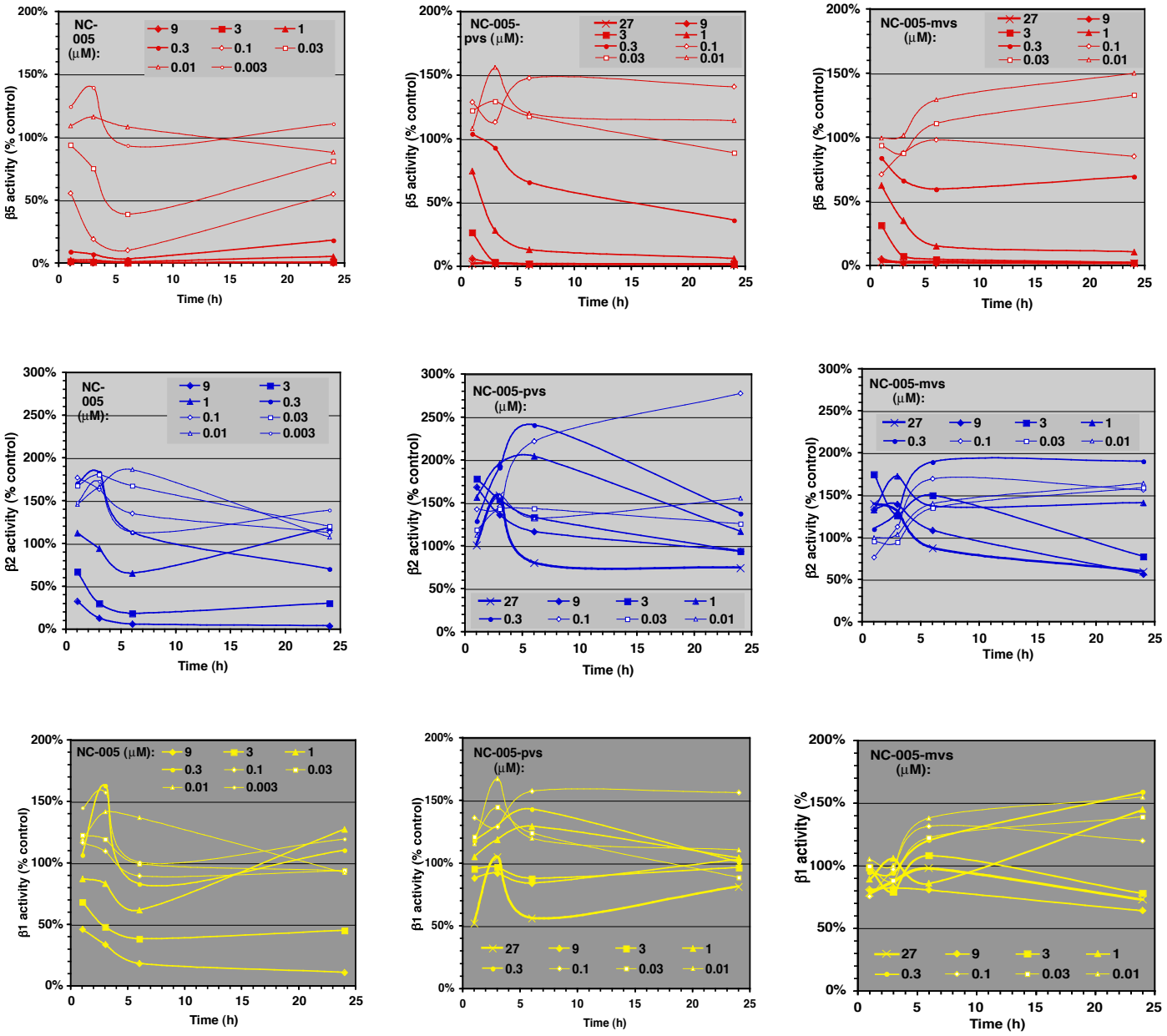

Figure S2. Inhibition of proteasome in HeLa S3 cells by NC-005, NC-005-pvs and NC-005-mvs.
HeLa S3 were continuously treated with inhibitors at concentrations indicated by the numbers in the legend. At time indicated an aliquot of culture was withdrawn, washed with PBS, and activities of individual active sites measured with ProteasomeGlo assay, and normalized to the number of cells. Mock-treated cells served as controls. Red lines, β5 sites; yellow lines, β1 sites; blue lines, β2 sites. Values are numbers ± S.E. of 2 or 3 independent experiments (biological replicates).

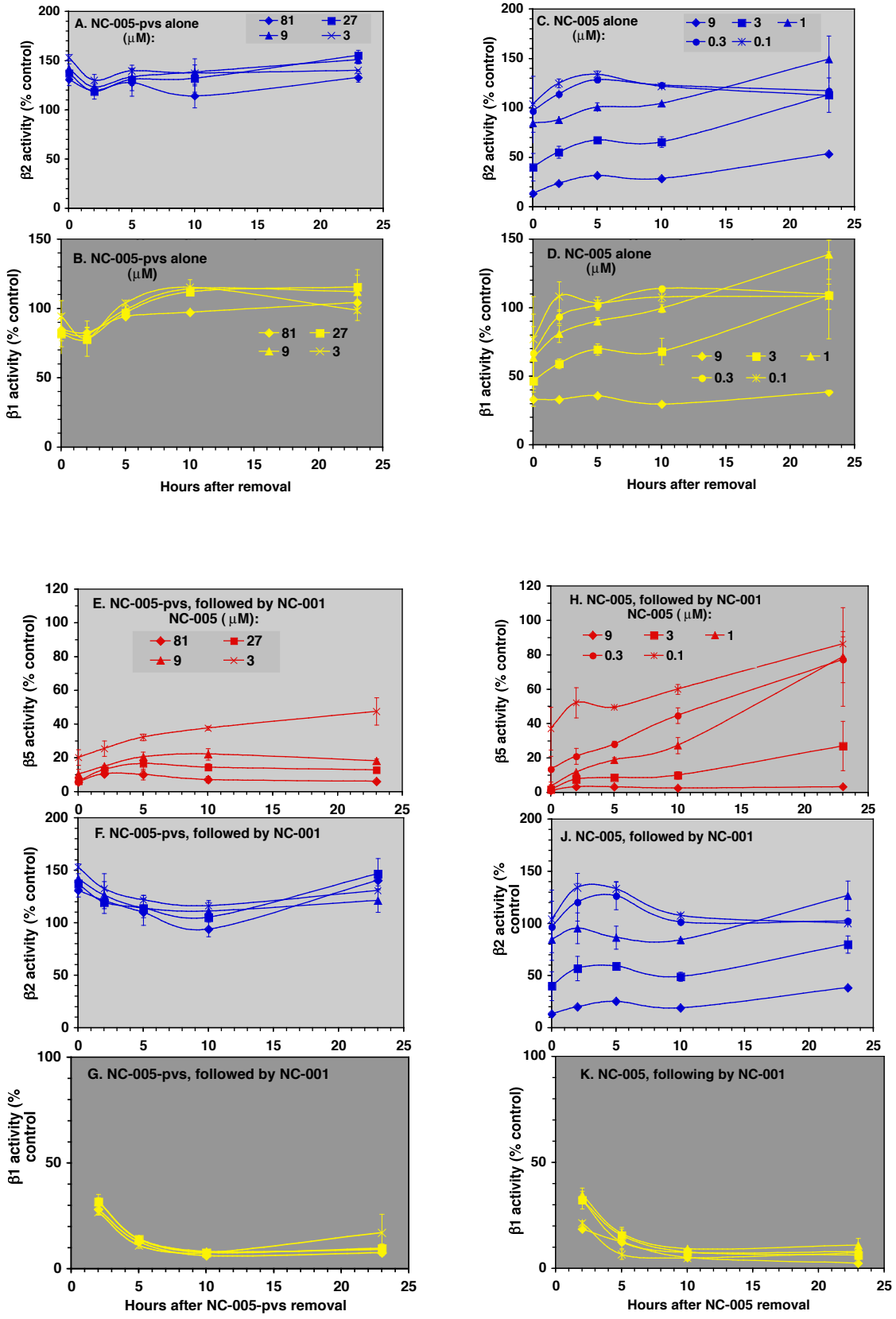

Figure S3. Additional data on proteasome activity in HeLa S3 cells from the 1h-treatment experiment shown on Fig. 4. A, B. β2 and β1 activity in cells treated with NC-005-pvs for 1 h in experiment shown on Fig. 4A. (See Fig. 4C for β5 activity.) C, D. β2 and β1 activity in cells treated with NC-005 for 1 h in experiment shown on Fig. 4B. (See Fig. 4D for β5 activity.) E-G. β5, β2, and β1 activity in cells treated with NC-001 after NC-005-pvs treatment in experiment shown on Fig. 4E. H-K. β5, β2, and β1 activity in cells treated with NC-001 after NC-005 treatment in experiment shown on Fig. 4F. Numbers in the legends are concentrations of NC-005-pvs and NC-005 used during 1 h treatment. Note that recovery rate of β5 activity in the presence of NC-001 is similar to recovery rate in its absence (Fig. 4C and 4D). NC-001 was used at 4 μM. β5 activity is in red, β2 activity is in blue, and β1 activity is in yellow. Numbers on the legends are concentrations (in μM) of NC-005-pvs and NC-005.