

General considerations on the study of YAP/TAZ regulation and activity in cell culture by 8xGTIIC lux and endogenous YAP/TAZ targets.

YAP and TAZ are transcriptional co-activators that integrate several inputs, including **soluble** cues from the cell environment/media (such as growth factor ligands and other molecules, such as LPA) as well as **insoluble** cues (rigidity of the plastic substrate, cell-cell contacts, cell-polarity, cell shape) (Cordenonsi et al., 2011; Azzolin et al., 2012; Wada et al., 2011; Dupont et al., 2011; Yu and Guan, 2012).

In particular, routine cell culturing conditions are potent upregulators of TAZ and YAP activity. An overarching positive input is the cell's spread shape and the fact that cells are plated on a very stiff substrate (i.e., the plastic of the tissue culture dish); under these conditions, TAZ is potently stabilized, and both TAZ and YAP are constitutively nuclear, as assayed by immunofluorescence.

In other words, please consider that transcriptional activity by YAP/TAZ is typically integral to tissue culture conditions, where YAP/TAZ are basally turned ON; and several cell lines may be actually addicted to such conditions, as attenuation of YAP/TAZ causes dramatic effects on growth and viability in the vast majority, if not all, the cell types so far tested. This also means that induction of 8xGTIIC-lux is profoundly different from other pathway-luciferase sensors (i.e., TOP-Flash for Wnt, or pCAGA12-lux for TGF β) as these are inactive – or actively repressed – in absence of the inducing stimuli. The same applies to endogenous Y/T targets, such as CTGF, CYR61, Ankd1 and others.

Although this basal YAP/TAZ activation is a very general phenomenon, its magnitude obviously also depends on the specific cell type; for example, we found that, in the breast cancer cell line MDA-MB-231, TAZ and YAP levels/activity are already at plateau levels. We thus use cell types like MDA-MB-231 for studies **whenever our scope is to oppose (basal) YAP/TAZ activation.**

Thus, a general consideration that may be taken into account when studying treatments that aim **to induce YAP/TAZ function** (i.e. the knockdown of a YAP/TAZ inhibitors, Wnt signaling and several others), is that the effect of such treatment may be blunted (or masked) by the above mentioned ‘background’ due to routine cell culturing conditions.

It follows that, whenever we want **to study YAP/TAZ upregulation** (for biological or biochemical experiments) in cultured cells, we minimize the basal ‘background’ of YAP/TAZ activity by allowing extensive cell-cell contacts (that turn OFF YAP/TAZ) in very confluent monolayers, at least for cells that do respond to contact-inhibition.

Another method used in the Yap/TAZ/Hippo field is to compare control and experimentally manipulated cells (i.e. by YAP/TAZ inhibiting siRNAs, or drug treatments) for different capacities to re-accumulate YAP/TAZ levels/activity after ‘releasing’ confluent monolayers through passaging, and reseeding cells them at lower densities.

Finally, a very effective mean to oppose YAP/TAZ activity is to seed cells on soft substrates: synthetic hydrogels, see Dupont et al., 2011, or a thick layer of Matrigel (from BD- Biosciences); note that simple coating the well with Matrigel is NOT sufficient to alleviate cell sensing of the underlying plastic and create a soft environment; or (only for biochemical experiments) to treat cells with the cytoskeletal inhibitor latrunculinA.